# Separation of Cations by Thiourea[1]

By Yasumitsu UZUMASA and Takeshi OKURA

(Received October 26, 1949)

The alkaline solution of thiourea, when boiled, decomposes and some sulfide ions are formed. The substance is also known to yield precipitates with some cations,[2] from which their sulfides are produced.[3] The authors found that such a property of thiourea makes it possible to separate the ions belonging to the copper sub-group from other cations.

**Thermal Decomposition of Thiourea in Aqueous Phase.**—The neutral solution of thiourea decomposes very gradually only when it is kept in boiling state for some time; but the decomposition takes place even at a comparatively lower temperature i. e., *ca.* 80° when the solution is made alkaline. For instance, when a solution prepared by adding 1 g. of thiourea to 200 cc. of 0.05 *N* sodium hydroxide was boiled gently for 5 minutes, the decomposition took place. It was found on qualitative test that the product contained, beside traces of carbonate-, sulfate-, sulfite- and nitrate-ions, a considerable amount of sulfide ion. The evolution of sulfur dioxide gas, though very little, was also recognized by its odor.

**The Amount of Thiourea Necessary for the Formation of Sulfide Precipitate.**—The ions which can be precipitated with the sulfide ion produced by the decomposition of thiourea in an alkaline medium are copper, cadmium, lead, mercury and bismuth, while other cations form no sulfide precipitate under the same condition. The boiling changes almost all sulfur in thiourea into sulfide ion, precipitating the metals of the copper sub-group quantitatively. This may be illustrated by the following example.

A series of copper sulfate solutions were prepared and buffered by a mixture of sodium chloride, sodium hydroxide and glycocol so that their *p*H was 11.29 at 10°. To each of the solutions containing 0.0144 g. of CuO were added different volumes of the thiourea solution[4] and they were made up to 40 cc. with distilled water respectively. The solution was then boiled gently for 2 minutes when a black precipitate was formed. The precipitate, after filtered through Toyo filter paper No. 5 A, was ignited to constant weight.

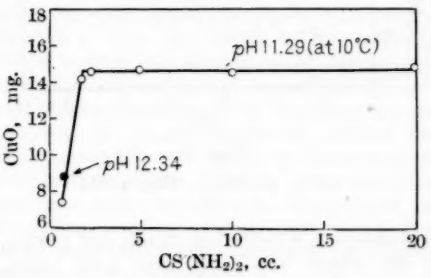


Fig. 1.

The results are summarized in Fig. 1 which shows that the precipitation of copper was complete when as much as 2 cc. of the thiourea solution was added. The theoretical amount of thiourea necessary for the quantitative separation of copper from the said solution is 1.67 cc., provided that all the sulfur in thiourea is available for the purpose. As illustrated in Fig. 1, the addition of 1.7 cc. of thiourea gave 0.0144 g. of CuO, the experimental results being in close agreement with the theoretical values.

The precipitate thus obtained is crystalline and adsorbs little foreign substances. It can be filtered easily with Toyo filter paper No. 5 A or even No. 1 etc., which have larger size of pores. This may be due to the fact that the velocity of precipitation is rather small and also that the precipitate once formed partly as hydroxide changes into sulfide very gradually.

**Influence of *p*H on Sulfide Formation by Thiourea.**—Thiourea does not produce sulfides in an acid medium. Our investigation has revealed that even when the solution is made alkaline, the precipitation is not complete unless $pH > 12$. This is confirmed by the following experiments.

To 20 cc. of the thiourea solution whose sulfur content was 3.5 mg. per cc. was added the buffer together with a solution containing copper or other ions of the sub-group. The total volume was made up to 55 cc., and the

(1) Read before the Hokkaido meeting of the Chemical Society of Japan, on July 31, 1949.

(2) J. H. Yoe and L. G. Overholser, *Ind. Eng. Chem., Anal. Ed.*, **14**, 435 (1942).

(3) Beilstein, Vol III, 180.

(4) 1 cc. of the solution contains 3.5 mg. of sulfur.

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solution was heated until it boiled gently for 2 minutes. The liquid was then cooled and filtered through Toyo filter paper No. 3. The precipitate was washed thoroughly and ignited to constant weight.[5] The results are shown in Fig. 2. The values are the means of several

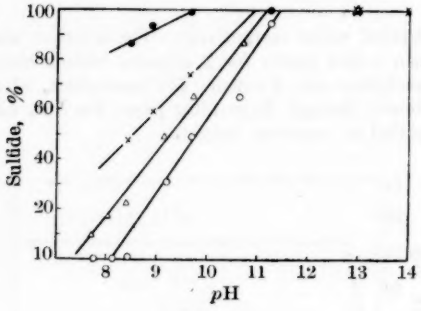


Fig. 2.

•, Cu; △, Pb; ×, Hg; ○, Cd

experments in which about 50 mg. and 100 mg. of the copper sub-group ions were taken. These data show that the velocity of sulfides precipitation depends upon *p*H of the system and also the concentration of thiourea as well as cations. In consequence, the best result should be expected when *p*H of the solution is kept 12.

**Separation of the Copper Sub-group Ions from Other Cations by Thiourea.**— The results obtained above suggest us a new means of separation of the copper sub-group ions from other cations. In case where $Cu^{++}$ and $Fe^{+++}$, for example, coexist in a solution, both metals precipitate as hydroxides when the solution is made alkaline. In presence of thiourea, $Cu^{++}$ changes into sulfide while ferric hydroxide remains as such. When a sufficient amount of acetic acid is added, the hydroxide dissolves on boiling[6] whereas the sulfide remains as precipitate. The experiments were carried out in the following way.

To a solution containing one of the copper group ions, e. g., $Cu^{++}$ as copper sulfate, was added three to five times as much another ion. Thiourea was present in the solution in such a ratio as $Cu : CS(NH_2)_2 = 1 : 5$ (in gram equivalent). Sodium hydroxide was added so that the solution was approximately 0.5 *N*. The mixture was heated and boiled for 2 minutes. After adding 10 cc. of acetic acid, the solution was allowed to stand on the water-bath for about 10 minutes. The precipitate was collected on Toyo filter paper No. 3, washed first with acetic acid and then with water and finally ignited to constant weight. The results are shown in Table 1.

Table 1

Influence of Coexisting Ions on the Precipitation of the Cu Sub-group Ions

| Co-existing ions | Cu Sub-group Ions | | | | |
|---|---|---|---|---|---|
| | Cu | Cd | Pb | Hg | Bi |
| Fe | 85.3% | 93.7% | 91.6% | 101% | 95.3% |
| Al | 92.8 | 96.4 | 96 | 83.2 | 96 |
| Co | 92.8 | 103.5 | 93.6 | 102.4 | 94.3 |
| Ni | 92.8 | 99.4 | 109 | 101.6 | 101 |
| Mn | 76.6 | 76.4 | 102.3 | 101.5 | 99 |
| Zn | 101.1 | 85.7 | 106 | 102.5 | 96 |
| Cr | 86.3 | 97.6 | 110 | 89 | 86 |

The values in the table are the separability of the copper sub-group ions from the coexisting ions which are found in the first column. They are the figures found with the solutions containing 0.05 g. and 0.1 g. of copper, cadmium and lead and also with the saturated solutions of mercury and bismuth.

Lead sulfide thus produced adheres to the inner wall of the vessel in which the reaction took place, forming a brilliant mirror. The deposit comes off easily from the wall in its early stage of formation. When the solution is allowed to stand for some time, however, the deposition becomes so firm that the mirror can be removed only on heating with nitric acid.

That the solubility to acetic acid of the sulfides obtained in this manner is very small is illustrated by the following experiments. A sulfide was precipitated in 50 cc. of the solution containing approximately 30 mg. of a copper sub-group ion, and the mixture, together with 30 cc. of acetic acid, was allowed to stand on the water-bath for 1 hour. The precipitate was then filtered and ignited to constant weight. The percentage by which the metal sulfides dissoved in acetic acid is as follows:

Cu 2.1%, Cd 2.8%, Pb 8.6%,[7] Hg 2.3%, Bi 4%

The separability of all the copper sub-group ions from the mixture of the ions of the third

(5) The mercury precipitate was dried at 110° and weighed.

(6) If the solution containing ferric hydroxide is boiled for some time, the hydroxide becomes less soluble.

(7) The error is considered to be large in case of lead because of its nature of adhering to the wall.

group as well as the fourth group was next studied. The experimental procedure is as follows.

To the mixture of copper, cadmium, lead, mercury and bismuth salts, the total amount of which was 0.1114 g. as sulfide, was added approximately five times as much $Fe^{+++}$, $Al^{+++}$, $Co^{++}$, $Ni^{++}$, $Mn^{++}$, $Zn^{++}$ and $Cr^{+++}$. The solution was made up to 100 cc. and treated with sodium hydroxide and thiourea in the same manner as in the previous experiments. It was boiled gently for 5 minutes and, after adding 50 cc. of acetic acid, allowed to stand on the water-bath for 10 minutes. The precipitate was then filtered and weighed. The separations of the copper sub-group from the fourth group, and the third group + the fourth group were also investigated. The results are summarized in Table 2.

Table 2

Separability of the Cu Sub-group Ions from Other Group Ions

| Co-existing ions | Separability |
|---|---|
| (1) the third group | 106.3% |
| (2) the fourth group | 103.4 |
| (3) {the third group and the fourth group} | 106.4 |
| (4) {tin sub-group, the third group and fourth group} | 168.3 |
| (5) {the same as (4), but the precipitate was washed with conc. NaOH} | 94 |

When the ions of the tin sub-group exist in the system, they precipitate as hydroxide insoluble in acetic acid, the separation being made difficult. In this case the sulfides are filtered, washed with acetic acid and treated with conc. sodium hydroxide solution. The precipitates of the tin sub-group metals then dissolve and a fairly good separation is performed. This is understood by the figures given at the bottom of the table.

It is thus confirmed that the ions of the copper sub-group can be separated nearly quantitatively from many other cations by thiourea. The present operation suggests us a new scheme of qualitative analysis without hydrogen sulfide which is under investigation in our laboratory. In such a plan, the effect of the excess of thiourea after the separation of copper sub-group ions should by no means be ignored. For instance, because of its reducing nature, thiourea, if present, positively retards the oxidation of $Mn^{++}$ to $MnO_2$ by means of nitric acid and potassium chlorate. The excess of thiourea must therefore be decomposed by evaporating up the solution with nitric acid to dryness before the solution is subjected to analysis of the fourth group.

A part of the expense of the investigation was defrayed from the research fund granted to one of the authors from the Hattori Hokokai, to which our hearty gratitude is expressed.

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# Dosage colorimétrique du chrome d'après la méthode au péroxyde d'argent et à la diphénylcarbazide

par Motoharu TANAKA

(Reçu le 22 Décembre, 1949)

En 1908, M. Moulin[1] a publié une méthode de dosage colorimétrique du chrome à l'aide du réactif organique diphénylcarbazide. Avant M. Moulin, M. Cazeneuve[2] avait déjà indiqué l'utilisation possible de ce réactif. La diphénylcarbazide amène la coloration de l'acide chromique en milieu acide, par conséquent il faut oxyder le chrome trivalent au chrome hexavalent. M. Moulin[3] a employé l'ébullition par l'eau oxygénée en présence d'un excès de potasse et Willard et Young[4] ont utilisé

(1) M. A. Moulin, *Bull. soc. chim., France*, (3), **31**, 295 (1908).

(2) P. Cazeneuve, *ibid.*, (3), **23**, 701 (1900).

(3) M. A. Moulin, *ibid.*, (3), **31**, 295 (1908).

(4) Willard et Young, *Ind. Eng. Chem.*, **20**, 769 (1928); Kolthoff et Sandell, *Ind. Eng. Chem., Anal. Ed.*, **2**, 140 (1930).

le persulfate d'ammonium ou le bromate de potassium, et l'adoption de la fusion avec le carbonate de soude permet d'oxyder le chrome dans l'analyse de la roche.[5]

Nous avons employé le réactif péroxyde d'argent en milieu d'acide sulfurique pour oxyder le chrome trivalent et cette méthode a fourni les resultats satisfaisants comme dans le cas du manganèse.[6]

## La condition d'oxydation et de coloration

Nous avons préparé la solution à 1 mg. Cr/cc. en dissolvant le chromate de potasse (Merck, pro analysi), que nous avons réduit ensuite par le gaz d'acide sulfureux, dont l'excès a été complètement chassé par le gaz d'acide carbonique. Cette solution a été utilisée dans l'état de dilution jusqu'à 1 γ Cr/cc.

Pour oxyder le chrome on a employé ce qu'on appelle le péroxyde d'argent,[7] que l'on peut facilement préparer par le moyen d'un mélange d'une solution de nitrate d'argent et de persulfate d'ammonium qui doit être agité. On a employé 0.10 à 0.15 g. de ce péroxyde d'argent en suspension dans l'eau à chaque dosage.

**La concentration de l'acide sulfurique pour l'oxydation.**—Les expériences ont été faites pour décider la concentration convenable pour arriver à l'oxydation du chrome par le péroxyde d'argent.

Table 1

| Concentration de l'acide sulfurique | moin de 1.5$N$ | 1.5$N$ | 3$N$ | 5$N$ | 8$N$ |
|---|---|---|---|---|---|
| Cr employé, γ | La déstruction de l'excès de péroxyde ne s'effectue que très lentement | 5.0$_0$ | 5.0$_0$ | 5.0$_0$ | 5.0$_0$ |
| Cr trouvé, γ | | 4.9$_6$ | 5.0$_0$ | 4.9$_9$ | 3.3$_5$ |

La table 1 indique que la concentration convenable se trouve entre 1.5 $N$ et 5 $N$ d'acide sulfurique. Nous avons pris la concentration 1.5 $N$, en tenant compte de ce que la coloration par la diphénylcarbazide est plus stable dans un milieu faiblement acide que dans un milieu fortement acide.

**La durée du chauffage.**—On ajoute le péroxyde d'argent à une solution froide contenant le chrome trivalent et puis l'excès de ce réactif est éliminé par chauffage au bain-marie. La durée du chauffage produit les effets suivants:

Table 2

| La durée du chauffage, minutes | 2 | 5 | 10 | 30 |
|---|---|---|---|---|
| Cr employé, γ | 5.0$_0$ | 5.0$_0$ | 5.0$_0$ | 5.0$_0$ |
| Cr trouvé, γ | 5.0$_0$ | 5.0$_2$ | 4.9$_7$ | 3.7$_2$ |

Sans doute, on peut conclure que 2 minutes de chauffage suffisent et qu'il ne faut dépasser 10 minutes pour éviter la réduction du chromate déjà oxydé.

**La stabilité de la couleur après la coloration.**—Le chrome oxydé par le péroxyde d'argent se combine immédiatement avec le réactif diphénylcarbazide, mais ce composé n'est pas tout à fait stable. On dit que sa stabilité varie selon la concentration de l'acide, dont nous avons pris environ 0.3 $N$. Pour examiner cette stabilité nous avons répété nos observations en variant des intervalles. Voici les résultats:

Table 3

| Le temps après la coloration, minutes | 3 | 10 | 20 | 30 | 40 |
|---|---|---|---|---|---|
| Cr employé, γ | 5.0$_0$ | 5.0$_0$ | 5.0$_0$ | 5.0$_0$ | 5.0$_0$ |
| Cr trouvé, γ | 5.0$_3$ | 4.9$_2$ | 4.9$_4$ | 5.0$_1$ | 6.0$_3$ |

Plus de 30 minutes après la coloration, une poudre brillante (dont la qualité n'est pas encore étudiée) apparaît parfois dans la solution colorée. Dans ce cas-là, le coefficient d'extinction obtenu devient d'un degré d'intensité supérieur à celui du chrome employé. Mais il n'y a aucune crainte de changement quand la comparaison est terminée dans les 30 minutes qui suivent la coloration.

**La quantité du réactif diphénylcarbazide.**—10 mg. de ce réactif est dissous dans un mélange de 2 cc. d'acétone et de 2 cc. d'eau. Cette solution est ajoutée dans une solution oxydée par le péroxyde d'argent. Les quantités différentes du réactif employé ne produisent aucun effet sur la coloration, comme la table 4 nous l'indique.

Table 4

| La quantité de solution de la diphénylcarbazide, cc. | 0.2 | 0.4 | 0.8 | 1.0 |
|---|---|---|---|---|
| Cr employé, γ | 5.0$_0$ | 5.0$_0$ | 5.0$_0$ | 5.0$_0$ |
| Cr trouvé, γ | 5.0$_0$ | 4.9$_9$ | 5.0$_2$ | 5.0$_5$ |

(5) E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.*, **8**, 336 (1936).

(6) Y. Murakami, *Bull. Chem. Soc. Japan.*, **22**, 159 (1949).

(7) La contribution à l'étude de ce péroxyde d'argent sera publiée par le même auteur.

## Le procédé recommandé

A 5 cc. de la solution à analyser contenant 1 cc. d'acide sulfurique (1 : 4), ajouter en agitant 0.10 à 0.15 g. de péroxyde d'argent, mettre la solution au bain-marie pendant environ 2 minutes jusqu'à la disparition du péroxyde d'argent pulvérisé et laisser reposer à la température ordinaire, ajouter de 0.2 cc. à 0.4 cc. de la solution de la diphénylcarbazide, diluer la solution à 25 cc. ou à 50 cc. au moyen d'eau distillée et comparer la coloration au bout de 10 minutes et pendant les10 minutes suivantes, en employant le colorimètre de Duboscq ou le photomètre de Pulrich.

## La courbe d'étalon

Quand on examine au spectrophotomètre la coloration de la chrome-diphénylcarbazide, dont le maximum d'absorption se trouve au voisinage de 5500 Å.,[8] on peut dire qu'il suffit d'employer le filtre S. 55 du photomètre de Pulfrich pour ce dosage. Cette coloration obéit à la loi de Lambert-Beer entre les concentrations 0.02 p.p.m. et 1 p.p.m. du chrome. Nous avons obtenu les coefficients d'extinction à diverses concentrations du chrome et nous avons tracé la courbe d'étalon suivante:

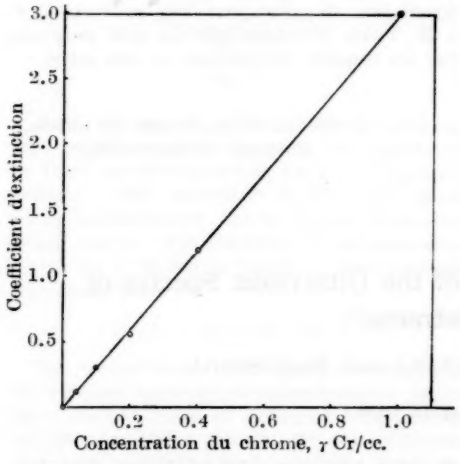


Fig. 1.—La courbe d'étalon. Filtre, S 55; Cuvette 50.00 mm.

La fonction entre les coefficients d'extinction et les concentration du chrome est faite au moyen de la méthode des moindres carrés.

$$C = 1.672\,E \quad (\gamma\ \text{Cr/cc.},\ \text{Cuvette 10 mm.}),$$

où $C$ est la concentration du chrome et $E$ le coefficient d'extinction.

## La reproductibilité de cette méthode

A diverses concentrations nous avons répété quelques expériences et étudié la reproductibilité de cette méthode.

Table 5

| La concentration du chrome | Cr employé, γ | Cr trouvé, γ | Ecart absolut, γ |
|---|---|---|---|
| 0.08 p.p.m. | $2.0_0$ | $2.0_2$ | $+ 0.0_2$ |
| 〃 | 〃 | $2.0_5$ | $+ 0.0_5$ |
| 〃 | 〃 | $1.9_9$ | $- 0.0_1$ |
| 0.2 p.p.m. | $5.0_0$ | $4.9_6$ | $- 0.0_4$ |
| 〃 | 〃 | $5.0_0$ | $0.0_0$ |
| 〃 | 〃 | $5.0_2$ | $+ 0.0_2$ |
| 0.4 p.p.m. | $10.0_0$ | $10.0_9$ | $+ 0.0_9$ |
| 〃 | 〃 | $9.9_8$ | $- 0.0_2$ |
| 〃 | 〃 | $10.0_8$ | $+ 0.0_8$ |
| 1 p.p.m. | $25.0_0$ | $24.9_2$ | $- 0.0_8$ |
| 〃 | 〃 | $24.8_1$ | $- 0.1_9$ |
| 〃 | 〃 | $25.0_9$ | $+ 0.0_9$ |

La table 5 nous montre que nous pouvons déterminer la teneur du chrome avec une précision fort satisfaisante.

## Influences des éléments étrangers

Les métaux comme cuivre, nickel, cobalt, mercure, cadmium, magnésium, qui produisent leur complexe coloré avec la diphénylcarbazide en milieu alcalin ou neutre, ne troublent pas ce procédé que l'on effectue dans le milieu acide.

La coloration du chrome par diphénylcarbazide est affaiblie par l'excès d'acide phosphorique ou de fluorure de sodium ajouté pour éliminer la couleur du fer. La coloration dûe au fer de l'ordre de 1 mg. peut être éliminée par l'addition de 0.2 cc. d'acide phosphorique ou de 30 mg. de fluorure de sodium, lesquels ne troublent pas le dosage.

On dit que la coloration dûe au molybdène s'affaiblit par l'addition d'acide oxalique.[8] Néanmoins, l'affaiblissement de la coloration du chrome est causé aussi par l'acide oxalique ajouté. Donc on ne peut éliminer la coloration dûe aux complexes, vanadium- et molybdène-diphénylcarbazide, qui sont très stables dans le milieu acide. Mais le molybdène de moins de 4 mg. et le vanadium de moins de 8 γ dans 25 cc. ne gênent pas ce dosage.

Les métaux comme fer, vanadium, et manganèse, qui sont en même temps oxydés par le péroxyde d'argent et qui produisent leur

(8) D. Brard, *Ann. chim. anal. chim. appl.*, **17**, 201 (1935).

composé coloré avec le réactif diphénylcarbazide, peuvent être séparés du chrome en utilisant de l'eau oxygénée et de la soude caustique. D'abord, on emploie l'excès de soude qui est neutralisé par l'acide sulfurique étendu jusqu'à *p*H 7.2. On peut séparer le chrome quantitativement du fer, du vanadium et du manganèse par deux ou trois précipitations. On utilise tout le filtrat pour l'analyse du chrome. La moitié du chrome dans le filtrat se trouve à l'état de hexavalent, mais l'oxydation par le péroxyde d'argent est nécessaire pour obtenir un résultat précis.

### Résultats de l'application aux échantillons naturels

L'échantillon ayant été décomposé par un certain acide, le chrome est précipité avec le fer par l'ammoniaque. Cette précipitation est dissoute dans l'acide sulfurique étendu et le chrome est séparé comme nous l'avons vu dans le chapitre précédant. Les résultats obtenus sont donnés ci-dessous:

Table 6

| | Echantillon | $Cr_2O_3$ % | |
|---|---|---|---|
| 1) | Jarosite | $5.8\times10^{-3}$ | |
| 2) | 1)+$7.3\times10^{-3}$% $Cr_2O_3$ | $12.9\times10^{-3}$ | calc. $13.1\times10^{-3}$ |
| 3) | Pyrolusite (a) | $0.5\times10^{-3}$ | |
| 4) | 〃 (b) | $0.0\times10^{-3}$ | |
| 5) | Basalte olivinique | $19.4\times10^{-3}$ | |
| 6) | Obsidienne dacitique à hyperthène | $0.7\times10^{-3}$ | |
| 7) | Basalte nephélinique | $18.5\times10^{-3}$ | |
| 8) | *Opuntia Ficus-indica Mill.* | $0.0\times10^{-3}$ | |


### Résumé

1. Nous proposons d'employer comme oxydant, qu'on appelle le péroxyde d'argent, pour oxyder le chrome que l'on détermine au moyen de la diphénylcarbazide. L'oxydation dans le milieu acide par le péroxyde d'argent s'effectue avec succès.

2. Nous avons fait des recherches sur l'influence des divers ions susceptibles de réagir avec la diphénylcarbazide et l'influence de ceux qui sont oxydés par le péroxyde d'argent.

3. Les résultats de l'analyse des minéraux, des roches et des plantes ont été donnés.

4. On peut appliquer cette méthode aux échantillons naturels très facilement et très simplement sans recourir à la fusion du carbonate de soude.

5. La déstruction complète de l'éxcès de l'oxydant et l'oxydation complète dans le milieu acide sont les points forts de cette méthode.


Qu'il soit permis à l'auteur, en terminant ce compte-rendu, de rendre un témoignage tout particulier de reconnaissance à ses maîtres M. Prof. Kenjiro Kimura, professeur à l'Université de Tokio et M. Kazuo Kuroda, professeur-adjoint à l'Université de Tokio, qui se sont donné tant de peine pour leur instruction, et à M. Yukio Murakami qui l'a aidé beaucoup par ses conseils très précieux et très utiles.

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# Molecular Orbital Treatment of the Ultraviolet Spectra of Cycloöctatetraene[1]

By Yuji MORI, Ikuzo TANAKA and Shoji SHIDA

(Received May 15, 1950)

## 1. Introduction

The energy levels of cycloöctatetraene have been calculated in our laboratory by the HLSP method[2] assuming two different structures $D_{2d}$[3] and $D_{4d}$,[4] and this calculation has been

(1) Presented at the Annual Meeting of the Chemical Society of Japan, Kyoto, April 2, 1950.

(2) I. Tanaka and S. Shida, *Bull. Chem. Soc. Japan*, **23**, 54 (1950).

(3) H. S. Kaufman, I. Fanckuchen and H. Mark, *J. Chem. Phys.*, **15**, 414-15 (1947); H. S. Kaufman, I. Fanckuchen and H. Mark, *Nature*, **161**, 165 (1948); K. Hedberg and V. Schomaker, *Annual Meeting of Amer. Chem. Soc.*, (1949).

(4) O. Bastiaisen, O. Hassel and A. Langseth, *Nature*, **160**, 128 (1947); O. Bastiaisen and O. Hassel, *Acta chemica scandinavica*, **3**, 209(1949); E. P. Lippincoit, R. C. Lord and R. S. MacDonald, *J. Chem. Phys.*, **16**, 548 (1948).

compared with the experimental results on cyclooctatetraene, namely absorption spectra, magnetic susceptibility and thermochemical data.

The purpose of the present paper is to calculate the energy levels of the molecule from the standpoint of the Goeppert Mayer and Sklar's method,[5] assuming the symmetry of cyclooctatetraene as $D_{2d}$, and to show how this calculation is in agreement with the experimental results.

## 2. The Orbitals

Table 1

| | E | $C_2$ | $2iC_4=S_4$ | $2C'_2$ | $2\sigma d$ |
|---|---|---|---|---|---|
| $A_1$ | 1 | 1 | 1 | 1 | 1 |
| $A_2$ | 1 | 1 | 1 | −1 | −1 |
| $B_1$ | 1 | 1 | −1 | 1 | −1 |
| $B_2$ | 1 | 1 | −1 | −1 | 1 |
| E | 2 | −2 | 0 | 0 | 0 |
| $\Gamma$ | 8 | 0 | 0 | 0 | 0 |

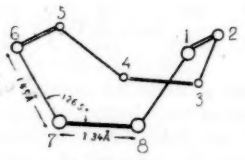


Fig. 1.

First, the wave functions of the $\pi$-electrons for cyclooctatetraene assuming the symmetry of $D_{2d}$[3] are determined by the LCAO approximation. The symmetry of the eight levels of cyclooctatetraene can be found from the group theory. The character of the symmetry group $D_{2d}$ is shown in Table 1. The reducible representation $\Gamma$ is

$$\Gamma = A_1 + A_2 + B_1 + B_2 + 2E.$$

The energy levels and their wave functions are obtained using the variation method. Since there are two kinds of distances between two neighboring carbon atoms, the two different resonance integrals (and similarly the two overlap integrals) must be distinguished from each other.

$$\left.\begin{aligned} &\int\tilde{\psi}_2 V_2 \psi_1 d\tau = \beta_d, \quad \int\tilde{\psi}_2\psi_1 d\tau = d_d, \\ &\int\tilde{\psi}_3 V_3 \psi_2 d\tau = \beta_s, \quad \int\tilde{\psi}_3\psi_2 d\tau = d_s, \end{aligned}\right\} \quad (1)$$

where $\psi_f$: the $2p\pi$ wave function for the $f$-th carbon atom,
$V_f$: the potential from the $f$-th carbon atom,

and

$$\int\tilde{\psi}_f\left(\sum_{l=1}^{8} V_l - V_f\right)\psi_f d\tau = \alpha. \quad (2)$$

Putting

$$x=(E_{2p}-E+\alpha)/\{(E_{2p}-E)d_d+\beta_d\}, \quad (3)$$

$$k=(E_{2p}-E)d_s+\beta_s/\{(E_{2p}-E)d_d+\beta_d\}, \quad (4)$$

where, $E_{2p}$ is the energy of a 2p electron in a carbon atom in the valence state. Then solving the secular determinant for $x$ we obtain

$$x=\pm(1+k), \quad \pm\sqrt{1+k^2}, \quad \pm(1-k). \quad (5)$$

Thus the wave functions are

$$\left.\begin{aligned}
\varphi_1 &= \frac{1}{\sqrt{8\sigma_1}}(\psi_1+\psi_2+\psi_3+\psi_4+\psi_5 \\
&\qquad +\psi_6+\psi_7+\psi_8), \\
\varphi_{+2} &= \frac{1}{\sqrt{8\sigma_2}}(\psi_1+\psi_2 e^{i\theta_1}+\psi_3 e^{i(\theta_1+\theta_2)} \\
&\qquad +\psi_4 e^{i(2\theta_1+\theta_2)}+\psi_5 e^{i2(\theta_1+\theta_2)} \\
&\qquad +\psi_6 e^{i(3\theta_1+2\theta_2)}+\psi_7 e^{i3(\theta_1+\theta_2)} \\
&\qquad +\psi_8 e^{i(4\theta_1+3\theta_2)}), \\
\varphi_{-2} &= \tilde{\varphi}_{+2}, \\
\varphi_3 &= \frac{1}{\sqrt{8\sigma_3}}(\psi_1+\psi_2-\psi_3-\psi_4+\psi_5 \\
&\qquad +\psi_6-\psi_7-\psi_8), \\
\varphi_4 &= \frac{1}{\sqrt{8\sigma_4}}(\psi_1-\psi_2-\psi_3+\psi_4+\psi_5 \\
&\qquad -\psi_6-\psi_7+\psi_8), \\
\varphi_{+5} &= \frac{1}{\sqrt{8\sigma_5}}(\psi_1+\psi_2 e^{i\theta_3}+\psi_3 e^{i(\theta_3+\theta_4)} \\
&\qquad +\psi_4 e^{i(2\theta_3+\theta_4)}+\psi_5 e^{i(2\theta_3+\theta_4)} \\
&\qquad +\psi_6 e^{i(3\theta_3+2\theta_4)}+\psi_7 e^{i3(\theta_3+\theta_4)} \\
&\qquad +\psi_8 e^{i(4\theta_3+3\theta_4)}), \\
\varphi_{-5} &= \tilde{\varphi}_{+5}, \\
\varphi_6 &= \frac{1}{\sqrt{8\sigma_6}}(\psi_1-\psi_2+\psi_3-\psi_4+\psi_5 \\
&\qquad -\psi_6+\psi_7-\psi_8),
\end{aligned}\right\} \quad (6)$$

where $\sigma_l$ is a normalizing factor, and

$$\left.\begin{aligned}
e^{i\theta_1} &= \frac{1+ik}{\sqrt{1+k^2}}, \quad e^{i\theta_2} = \frac{-1+ik}{\sqrt{1+k^2}}, \quad \theta_1+\theta_2=\frac{\pi}{2}, \\
e^{i\theta_3} &= \frac{k+i}{\sqrt{1+k^2}}, \quad e^{i\theta_4} = \frac{-k+i}{\sqrt{1+k^2}}, \quad \theta_3+\theta_4=\frac{3\pi}{2}.
\end{aligned}\right\} \quad (7)$$

These wave functions are

(5) G. Mayer and A. L. Sklar, *J. Chem. Phys.*, **6**, 645 (1938).

$$\varphi_1 \subset A_1, \quad \varphi_{\pm 2} \subset E, \quad \varphi_3 \subset B_2,$$
$$\varphi_4 \subset B_1, \quad \varphi_{\pm 1} \subset E, \quad \varphi_6 \subset A_2.$$

When $k = 1$, the wave functions become

$$\varphi_l = \frac{1}{\sqrt{8}\sigma_l} \sum_{f=1}^{8} \psi_f \exp[2\pi i f l/8], \quad l = 0, \pm 1, \pm 2, \pm 3, 4, \tag{8}$$

which are the same as those of Hückel.[5] These wave functions appear when the symmetry of cycloöctatetraene is assumed as $D_{4d}$ or $D_{8h}$.

The antisymmetrized molecular orbitals of 8-electron system for the ground state and the lower excited states are

$$\Phi_l = \frac{1}{\sqrt{8!}} \sum_P (-1)^P P \Psi_l \chi_k, \tag{9}$$

where for the ground state

$$\Psi_0 = \varphi_1(1)\varphi_1(2)\varphi_2(3)\varphi_2(4) \times \varphi_{-2}(5)\varphi_{-2}(6)\varphi_3(7)\varphi_3(8), \tag{10}$$

for the excited states

$$\Psi_1 = \varphi_1(1)\varphi_1(2)\varphi_2(3)\varphi_2(4) \times \varphi_{-2}(5)\varphi_{-2}(6)\varphi_3(7)\varphi_4(8), \tag{11}$$

$$\Psi_2 = \varphi_1(1)\varphi_1(2)\varphi_2(3)\varphi_2(4) \times \varphi_{-2}(5)\varphi_{-2}(6)\varphi_4(7)\varphi_4(8), \tag{12}$$

$$\left.\begin{aligned} \Psi_3 &= \varphi_1(1)\varphi_1(2)\varphi_2(3)\varphi_2(4) \times \varphi_{-2}(5)\varphi_{-2}(6)\varphi_3(7)\varphi_{+5}(8), \\ \text{or} \quad &= \varphi_1(1)\varphi_1(2)\varphi_2(3)\varphi_2(4) \times \varphi_{-2}(5)\varphi_{-2}(6)\varphi_3(7)\varphi_{-5}(8), \end{aligned}\right\} \tag{13}$$

$$\left.\begin{aligned} \Psi_4 &= \varphi_1(1)\varphi_1(2)\varphi_2(3)\varphi_2(4) \times \varphi_3(5)\varphi_3(6)\varphi_{-2}(7)\varphi_4(8), \\ \text{or} \quad &= \varphi_1(1)\varphi_1(2)\varphi_{-2}(3)\varphi_{-2}(4) \times \varphi_3(5)\varphi_3(6)\varphi_2(7)\varphi_4(8), \end{aligned}\right\} \tag{14}$$

and from the group theory

$$\Psi_0 \subset A_1, \ \Psi_1 \subset A_2, \ \Psi_2 \subset A_1, \ \Psi_3 \subset E, \ \Psi_4 \subset E.$$

$\chi_k$, spin function, is for the singlet states

$$\left.\begin{aligned} \chi_s &= \alpha(1)\beta(2)\alpha(3)\beta(4)\alpha(5) \times \beta(6)\alpha(7)\beta(8), \\ \text{or} \quad &= \alpha(1)\beta(2)\alpha(3)\beta(4)\alpha(5)\beta(6) \times \frac{1}{\sqrt{2}}\{\alpha(7)\beta(8) - \beta(7)\alpha(8)\}, \end{aligned}\right\} \tag{15}$$

and for the triplet states

$$\left.\begin{aligned} \chi_t &= \alpha(1)\beta(2)\alpha(3)\beta(4)\alpha(5) \times \beta(6)\alpha(7)\alpha(8), \\ \text{or} \quad &= \alpha(1)\beta(2)\alpha(3)\beta(4)\alpha(5) \times \beta(6)\beta(7)\beta(8), \\ \text{or} \quad &= \alpha(1)\beta(2)\alpha(3)\beta(4)\alpha(5)\beta(6) \times \frac{1}{\sqrt{2}}\{\alpha(7)\beta(8) + \beta(7)\alpha(8)\}. \end{aligned}\right\} \tag{16}$$

Thus the energy values for these states will be calculated in Section 3 including electronic interaction.

## 3. Calculations

The average values of Hamiltonian are given by

$$E_l = \int \tilde{\Phi}_l H \Phi_l \, dV. \tag{17}$$

The Hamiltonian H is

$$H = \sum_{\nu=1}^{8} \left( -\frac{h^2}{8\pi^2 m} \Delta_\nu + \sum_{f=1}^{8} V_{f\nu} \right) + \sum_{\substack{\nu=1 \\ \nu<\mu}}^{8} \frac{e^2}{r_{\nu\mu}}, \tag{18}$$

where $\nu$ and $\mu$ show the number of a $\pi$-electron, $f$ denotes the number of a carbon atom, and $V_{f\nu}$ is the potential of a neutral carbon atom[5] minus the potential of a $2p\pi$ electron.

The energies, including electronic interaction, are for the ground state

$$(^1A_1) \quad {}^1E_0 = 2\varepsilon_1 + 4\varepsilon_2 + 2\varepsilon_3 + \gamma_{11} + 8\gamma_{12} + 4\gamma_{13} + 6\gamma_{22} + 8\gamma_{23} + \gamma_{33} - 4\delta_{12} - 2\delta_{-22} - 2\delta_{13} - 4\delta_{23}, \tag{19}$$

for the excited singlet states

$$\left.\begin{aligned} (^1A_2) \quad {}^1E_1 &= 2\varepsilon_1 + 4\varepsilon_2 + \varepsilon_3 + \varepsilon_4 + \gamma_{11} + 8\gamma_{12} + 2\gamma_{13} + 2\gamma_{14} + 6\gamma_{22} + 4\gamma_{23} + 4\gamma_{24} + \gamma_{34} - 4\delta_{12} - 2\delta_{-22} - \delta_{13} - \delta_{14} - 2\delta_{23} - 2\delta_{24} + \delta_{34}, \\ (^1A_1) \quad {}^1E_2 &= 2\varepsilon_1 + 4\varepsilon_2 + 2\varepsilon_4 + \gamma_{11} + 8\gamma_{12} + 4\gamma_{14} + 6\gamma_{22} + 8\gamma_{24} + \gamma_{44} - 4\delta_{12} - 2\delta_{-22} - 2\delta_{14} - 4\delta_{24}, \\ (^1E) \quad {}^1E_3 &= 2\varepsilon_1 + 4\varepsilon_2 + \varepsilon_3 + \varepsilon_5 + \gamma_{11} + 8\gamma_{12} + 2\gamma_{13} + 2\gamma_{15} + 6\gamma_{22} + 4\gamma_{23} + 4\gamma_{25} + \gamma_{35} - 4\delta_{12} - \delta_{13} - \delta_{15} - 2\delta_{-22} - 2\delta_{23} - \delta_{-25} - \delta_{25} + \delta_{35}, \\ (^1E) \quad {}^1E_4 &= 2\varepsilon_1 + 3\varepsilon_2 + 2\varepsilon_3 + \varepsilon_4 + \gamma_{11} + 6\gamma_{12} + 4\gamma_{13} + 2\gamma_{14} + 3\gamma_{22} + 6\gamma_{23} + 3\gamma_{24} + \gamma_{33} + 2\gamma_{34} - 3\delta_{12} - 2\delta_{13} - \delta_{14} - \delta_{-22} - 3\delta_{23} - \delta_{34}, \end{aligned}\right\} \tag{20}$$

(6) E. Hückel, *Zeits. Physik*, **70**, 204 (1931).

and for the triplet states

$$ {}^3\mathbf{E}_1={}^1\mathbf{E}_1-2\delta_{34},\quad {}^3\mathbf{E}_3={}^1\mathbf{E}_3-2\delta_{35},\quad {}^3\mathbf{E}_4={}^1\mathbf{E}_4-2\delta_{24}. \tag{21} $$

The integrals, $\varepsilon_i$, $\gamma_{ij}$ and $\delta_{ij}$ in these expressions are

$$ \varepsilon_i=\int\tilde{\varphi}_i(\nu)\Big(-\frac{h^2}{8\pi^2m}\triangle_\nu+\sum_{f=1}^{8}\mathbf{V}_{f\nu}\Big)\varphi_i(\nu)\,\mathrm{d}\tau_\nu, \tag{22} $$

$$ \gamma_{ij}=\iint\frac{\mathrm{e}^2}{r_{\nu\mu}}|\varphi_i(\nu)|^2|\varphi_j(\mu)|^2\,\mathrm{d}\tau_\nu\mathrm{d}\tau_\mu, \tag{23} $$

$$ \delta_{ij}=\iint\frac{\mathrm{e}^2}{r_{\nu\mu}}[\varphi_i(\nu)\varphi_j(\mu)]\,\varphi_j(\nu)\varphi_i(\mu)\mathrm{d}\tau_\nu\mathrm{d}\tau_\mu. \tag{24} $$

Then, $\varepsilon_i$, $\gamma_{ij}$ and $\delta_{ij}$ are expanded in terms of $A_i$, $B_i$, $B'_i$, $R_i$, $C_i$, Q, $d_i$, for instance,[7]

$$ \left.\begin{aligned}
\varepsilon_2&=\mathrm{E}_{2\mathrm{p}}+\frac{1}{\sigma_2}\Big[-\mathbf{A}'-\mathrm{Q}-\frac{1}{S}\mathrm{R}_1-\frac{k}{S}\mathrm{R}_7\\
&\quad-\frac{1}{S}\Big\{\mathrm{B}_1+2(\mathrm{B}_3+\mathrm{B}_5+\mathrm{B}_7)\Big\}\\
&\quad-\frac{k}{S}\Big\{\mathrm{B}_3+2(\mathrm{B}_2+\mathrm{B}_4+\mathrm{B}_6)\Big\}\Big],\\
\gamma_{33}&=\frac{1}{8\sigma_3^2}\Big\{\mathbf{A}+4(\mathrm{B}_1+\mathrm{B}_3+\mathrm{B}_5+\mathrm{B}_7)\\
&\quad-4(\mathrm{B}_2+\mathrm{B}_4+\mathrm{B}_6+\mathrm{B}_8)-8\mathrm{B}'_2\\
&\quad+4\mathrm{B}'_3+2\mathrm{B}'_5+2\mathrm{C}_1+2\mathrm{C}_7\Big\},\\
\delta_{34}&=\frac{1}{8\sigma_3\sigma_4}\Big\{\mathrm{A}_0-\mathrm{A}_1-\mathrm{A}_7+2\mathrm{A}_2\\
&\qquad-\mathrm{A}_3-\mathrm{A}_5+\mathrm{A}_4\Big\},\\
\sigma_2&=1+\frac{1}{S}\mathrm{d}_1+\frac{k}{S}\mathrm{d}_7,\\
\sigma_3&=1+\mathrm{d}_1-\mathrm{d}_7-2\mathrm{d}_2,
\end{aligned}\right\} \tag{25} $$

where

$$ \left.\begin{aligned}
&\mathrm{A}_i=\iint\frac{\mathrm{e}^2}{r_{\nu\mu}}\psi_1(\nu)^2\psi_{i+1}(\mu)^2\mathrm{d}\tau_\nu\mathrm{d}\tau_\mu,\\
&\sum_{i=0}^{7}\mathrm{A}_i=\mathbf{A},\quad \sum_{i=1}^{7}\mathrm{A}_i=\mathbf{A}',\\
&\mathrm{B}_i=\iint\frac{\mathrm{e}^2}{r_{\nu\mu}}\psi_1(\nu)^2\\
&\qquad\times\psi_i(\mu)\psi_{i+1}(\mu)\mathrm{d}\tau_\nu\mathrm{d}\tau_\mu,\\
&\mathrm{B}_i'=\iint\frac{\mathrm{e}^2}{r_{\nu\mu}}\psi_1(\nu)\psi_2(\nu)\psi_i(\mu)\\
&\qquad\times\psi_{i+1}(\mu)\mathrm{d}\tau_\nu\mathrm{d}\tau_\mu,\\
&\mathrm{C}_i=\iint\frac{\mathrm{e}^2}{r_{\nu\mu}}\psi_1(\nu)\psi_{i+1}(\nu)\psi_1(\mu)\\
&\qquad\times\psi_{i+1}(\mu)\mathrm{d}\tau_\nu\mathrm{d}\tau_\mu,\\
&\mathrm{R}_i=\int\psi_{i+1}(\nu)\,\mathbf{H}_{i+1}(\nu)\psi_1(\nu)\mathrm{d}\tau_\nu,\\
&\mathrm{Q}=\int\psi_1(\nu)\big(\mathbf{H}_2(\nu)+\mathbf{H}_3(\nu)\big)\psi_1(\nu)\mathrm{d}\tau_\nu,\\
&\mathrm{d}_i=\int\psi_1(\nu)\psi_{i+1}(\nu)\mathrm{d}\tau_\nu.
\end{aligned}\right\} \tag{26} $$

$\mathbf{H}_i(\nu)$ is the potential of a neutral carbon atom.[5] The $2p\pi$ orbital of a carbon atom is

$$ \psi_{2p\pi}(r.\theta.\varphi)=\sqrt{\frac{Z^5}{32\pi}}r\sin\theta\cos\varphi\exp[-Zr/2], \tag{27} $$

where $Z$ is the effective nuclear charge 3.18, calculated by Zener.[8] The numerical values of $A_i$, $B_i$, $B'_i$, $R_i$, $C_i$, Q, $d_i$[9] are determined from the values of Parr and Crawford's calculation.[10]

The results of calculation are shown in Table 2 and 3.

Table 2

| | | | | | |
|---|---|---|---|---|---|
| $A_0=$ | 16.94 e.V. | $B_3=$ | 1.39 e.V. | $C_1=$ | 1.11 e.V. |
| $A_1=$ | 9.31 〃 | $B_4=$ | 0.56 〃 | $C_7=$ | 0.78 〃 |
| $A_2=A_6=$ | 5.61 〃 | $B_5=$ | 1.23 〃 | $R_1=$ | 2.12 〃 |
| $A_3=$ | 4.63 〃 | $B_6=$ | 0.66 〃 | $R_7=$ | 0.88 〃 |
| $A_4=$ | 4.33 〃 | $B_7=$ | 1.81 〃 | Q = | 1.66 〃 |
| $A_5=$ | 4.78 〃 | $B_8=$ | 1.84 〃 | $d_1=$ | 0.28 |
| $A_7=$ | 8.60 〃 | $B'_2=$ | 0.36 〃 | $d_7=$ | 0.13 |
| $B_1=$ | 3.60 〃 | $B'_3=$ | 0.46 〃 | $d_2=$ | 0.02 |
| $B_2=$ | 0.92 〃 | $B'_5=$ | 0.36 〃 | $k$ = | 0.44 |

(7) In convenience we put $k=\frac{\beta_s}{\beta_d}$ and $S=\sqrt{1+k^2}$.

(8) Zener, *Phys. Rev.*, **36**, 51 (1930).

(9) The integral $\iint\frac{\mathrm{e}^2}{r_{\nu\mu}}\phi_a(\nu)\phi_b(\nu)\phi_c(\mu)\phi_d(\mu)\mathrm{d}\tau_\nu\mathrm{d}\tau_\mu$ was approximated by $\mathrm{d}_{ab}\cdot\mathrm{d}_{cd}\times\iint\frac{\mathrm{e}^2}{r_{\nu\mu}}\mathrm{K}_{ab}(\nu)^2\cdot\mathrm{K}_{cd}(\mu)^2\mathrm{d}\tau_\nu\mathrm{d}\tau_\mu$.
Rf. A. London, *J. Chem. Phys.*, **13**, 417 (1945).

(10) R. G. Parr and B. L. Crawford, *J. Chem. Phys.*, **16**, 1049 (1948); *cf.* C. C. J. Roothaan and R. G. Parr, *J. Chem. Phys.*, **17**, 1001 (1949).

Table 3

| | | |
|---|---|---|
| $\gamma_{12}$= 7.16 e.V. | $\gamma_{33}$= 7.63 e.V. | $\delta_{25}$= 1.31 e.V. |
| $\gamma_{13}$= 7.38 〃 | $\gamma_{44}$= 8.97 〃 | $\delta_{34}$= 0.72 〃 |
| $\gamma_{14}$= 7.73 〃 | $\delta_{12}$= 2.37 e.V. | $\delta_{35}$= 1.39 〃 |
| $\gamma_{15}$= 7.35 〃 | $\delta_{13}$= 1.44 〃 | |
| $\gamma_{22}$= 7.37 〃 | $\delta_{14}$= 1.49 〃 | $\varepsilon_1$= $E_{2p}$−45.57 e.V. |
| $\gamma_{23}$= 7.64 〃 | $\delta_{15}$= 1.04 〃 | $\varepsilon_2$= $E_{2p}$−46.38 〃 |
| $\gamma_{24}$= 7.87 〃 | $\delta_{-22}$=1.50 〃 | $\varepsilon_3$= $E_{2p}$−46.85 〃 |
| $\gamma_{25}$= 7.52 〃 | $\delta_{23}$= 1.94 〃 | $\varepsilon_4$= $E_{2p}$−45.73 〃 |
| $\gamma_{33}$= 8.03 〃 | $\delta_{24}$= 1.58 〃 | $\varepsilon_5$= $E_{2p}$−41.00 〃 |
| $\gamma_{34}$= 7.84 〃 | $\delta_{-25}$=0.85 〃 | |

## 4. Considerations

The energy levels of the ground state and the lower excited states of cycloöctatetraene are shown in Fig. 2. The ground state is the singlet state of $^1A_1$, and then this is in agreement with the experimental result that the magnetic susceptibility of cycloöctatetraene is diamagnetic. The intensity of the shoulder of the absorption curve[11] (max. 2800 Å.) is relatively weak, so this absorption band seems to be due to a forbidden transition.

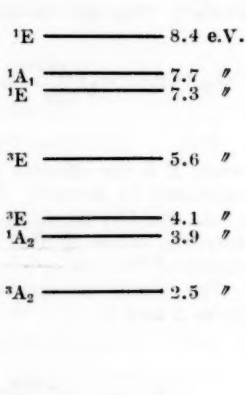


Fig. 2.

The transitions from the ground state $^1A_1$ to the excited states $^1E$ are allowed. According to our calculation, the wave lengths for these transitions are 1700 Å. (7.3 e.V.) and 1470 Å. (8.4 e.V.). The $^1A_1 \sim {}^1A_2$ forbidden transition is 3.9 e.V. (about 3100 Å.) and the $^1A_1 \sim {}^1A_1$ forbidden transition is 7.7 e.V. (about 1600 Å). The results of the calculation by the HLSP method[2] show that the $^1A_1 \sim {}^1E$ transition is about 2100 Å. and the $^1A_1 \sim {}^1A_1$ forbidden transition is about 2800 Å. band. So the strong band below 2000 Å. may be due to the $^1A_1 \sim {}^1E$ transition and the 2800 Å. weak band to the $^1A_1 \sim {}^1A_1$ or the $^1A_1 \sim {}^1A_2$ transition.

The energy levels of cycloöctatetraene are also calculated by the authors, as having the symmetry of $D_4$, in a similar procedure to that in the case of $D_{2d}$ by the GMS method. But the results calculated assuming as $D_4$ are not in agreement with the observed values of absorption spectra and magnetic properties.

## 5. Summary

The energy levels of cycloöctatetraene as $D_{2d}$ were calculated byt he GMS method. Then it was shown by the authors that the ground state of cycloöctatetraene may be diamagnetic, and that the wave length of the absorption spectra calculated assuming as $D_{2d}$ is approximately in agreement with the expetimental results.

The authors' thanks are due to the Ministry of Education for the financial grant.

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(11) S. Miyagawa and I. Tanaka, unpublished.

# Effects of Hydrochloric Acid on the Paper Chromatography of Amino Acids[1]

By Shin-ichi Ishii[2] and Toshio Ando[2]

(Received June 19, 1950)

Paper partition chromatography has been proved to be a useful and convenient tool for

(1) The present paper was read by one of the authors (S. I.) before the 3rd Annual Meeting of the Chemical Society of Japan in Kyoto held on April 1, 1950. A part of the work has been reported in the Report of the Radiation Chemistry Research Institute, the University of Tokyo, 5, 53 (1950).

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amino acid analysis.[3,4,5,6] Rf values of amino acids, the specific characters showing the ratios of the distances reached by them to those by the developing solvents, have been determined in their aqueous solutions developed by several solvents, and applied to the identification of each of the amino acids. However, in practice, analysis by this method of the solution containing hydrochloric acid, especially in the hydrolyzed mixture of protein or peptide with hydrochloric acid, is very often carried out in laboratories. Repeated treatments of such solution by evaporating to dryness, with successive addition of water, can never remove hydrochloric acid completely from the solution, a quantity of the acid corresponding to the concentration of *ca.* 1 *N* being retained, although this obviously depends on the concentration of amino acids. Neutralization of the acid with alkali was often tried, but an undesirable effect of the salt produced upon the chromatogram may not then be neglected.[7] The treatment, moreover, is of very troublesome and fast impracticable in case of treating a minute volume of solution, a case often met with in the paper chromatographic method. It has never been studied whether the presence of hydrochloric acid may affect the Rf values of amino acids or not, though recently H. B. Bull *et al.*[8] and S. Aronoff[9] observed the Rf values of amino acids in the solutions of some hydrogen ion concentrations, using phenol as a solvent. The former investigators found no difference between those in neutral and alkaline or acidic solutions, while the latter found a considerable effect of hydrogen ion concentrations on the chromatogram of lysine in alkaline solution, but neither of the investigations has been extended to the range of higher acidity. As a preliminary test proved that the effect of hydrochloric acid on the chromatograms was sometimes so remarkable in cases of some solvents, the present systematic observation was carried out in order to clarify the effect in cases of several amino acids, solvents and concentrations of hydrochloric acid

## Experimental Method

Amino acids used in the experiment were L-leucine, DL-valine, DL-alanine, glycine, L-arginine monohydrochloride and L-aspartic acid. Each of them was dissolved individually in 1% concentration, except L-leucine and DL-valine (both in 0.5%), in hydrochloric acid of various concentrations (0—12 *N*). A definite small volume from the solution was applied to a marked spot on each of the paper strips (the Tôyo filter paper, No. 2, 2×40 cm.). After the strip was allowed to stand in the room for about twenty hours, the chromatogram of the one-dimensional type, descending or ascending, was made as usual. As solvents, *n*-butanol, isovalerianic acid, *n*-butanol-acetic acid, phenol and collidine-lutidine were used. These were saturated with water except *n*-butanol-acetic acid and phenol. *n*-Butanol-acetic acid was composed of 4 volumes of *n*-butanol, 1 volume of acetic acid and 1 volume of water. Phenol was used as 80% (W/W) aqueous solulion.

After development and drying, the spot of amino acid was revealed by spraying with 0.2% solution of ninhydrin in water-saturated butanol followed by heating. Subsequently, the diffused area of hydrochloric acid on the strip was also revealed by spraying with 0.04% solution of bromophenol blue (B.P.B.) or thymol blue (T.B.) in 95% ethanol. The chromatograms obtained were compared with one another concerning each solvent, the results of which are as follows.

## Results and Discussions

***n*-Butanol.**—In case *n*-butanol was used as a solvent, hydrochloric acid was found to

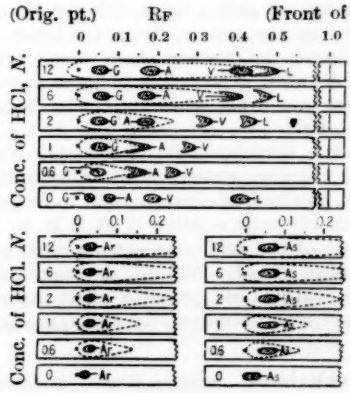


Fig. 1.—Chromatogram of amino acids in hydrochloric acid of various concentrations developed by *n*-butanol: Temperaure, 5-10°; development, descending; L, leucine; V, valine; A, alanine; G, glycine; Ar, arginine; As, aspartic acid. Areas surrounded by broken lines mean those of diffused hydrochloric acid. Chromatograms for corresponding concentrations of the acids were shown together in the same figures respectively, although experiments on each amino acid were carried out independently.

(3) R. Consden, A.H.Gordon and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).
(4) R. Consden, *Nature*, **162**, 359 (1948).
(5) A. J. P. Martin, *Ann. N. Y. Acad. Sci.*, **49**, 249 (1948); *Ann. Repts. Progress Chem.*, **45**, 267 (1949).
(6) D. L. Clegg, *Anal. Chem.*, **22**, 48 (1950).
(7) A. H. Gordon, *Nature*, **162**, 180 (1948).
(8) H. B. Bull, J. W. Hahn and V. H. Baptist, *J. Am. Chem. Soc.*, **71**, 550 (1949).
(9) S. Aronoff, *Science*, **110**, 590 (1949).

increase the $R_F$ value of each amino acid with an increase in the concentration of hydrochloric acid. The results were shown in Fig. 1. In extreme cases, where the concentration of hydrochloric acid was 12 *N* or 6 *N*, the position of valine and alanine was moved to the usual one of leucine and valine respectively. Accordingly, the identification of these amino acids may possibly be often mistaken without the knowlegde of this fact.

The diffused area of hydrochloric acid revealed by B.P.B. spread more extensively as the concentration of the acid increased, though its size was independent of the kind of amino acids. The area was shown surrounded by broken lines in the figure. Hydrochloric acid seemed to be accompanied thus far with *n*-butanol as a result of its considerable solubility in the latter. The reason why each amino acid travels abnormally for a longer distance may be understood from the fact that amino acid is more soluble in *n*-butanol containing hydrochloric acid than in it alone. The relation between the acid areas and the location of amino acids was fairly interesting in this case. The spots of amino acids of higher $R_F$ values went just ahead of the field of hydrochloric acid, forming a distinct saddle shape colored by ninhydrin as strongly as usual. The further the areas of hydrochloric acid extended, the further the spots of amino acids were pushed forward with them. When the formers went too far for the amino acids of lower $R_F$ values to ride on the top of them, the latters were left behind the formers and were included inside of them. The shapes of them were then changed into ovals, while their color became a little paler.

When a chromatogram of a sample containing an unknown concentration of hydrochloric acid is obtained, the paper ought to be sprayed with an alcoholic solution of B. P. B. to show the area of the acid. On comparing this with the figures already obtained, the concentration of the acid and its probable effect upon the chromatogram therefore may also approximately be supposed.

**Isovalerianic acid.**—When isovalerianic acid was used as a solvent, contrary to the case of *n*-butanol, hydrochloric acid was found to decrease remarkably the $R_F$ value and also the strength of coloring of each amino acid, as shown in Fig. 2, with an increase in the concentration of hydrochloric acid. The diffused area of hydrochloric acid revealed by T. B. was now limited to the neighborhood of the original point and did extend no more. The fact seems to be resulted from the difficult solubility of hydrochloric acid in isovalerianic acid, contrary to that in *n*-butanol. Hydrochloic acid thus retained in the stationary phase near the original point may likely prevent the amino acids from moving.

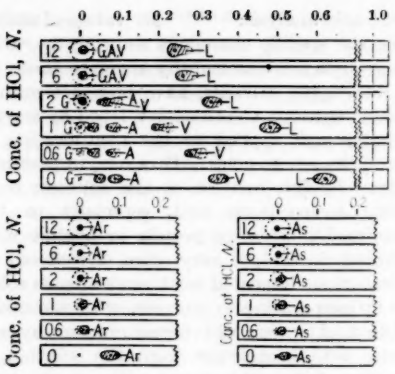


Fig. 2.—Chromatograms of amino acids in hydrochloric acid of various concentrations developed by isovalerianic acid: Temperature, 5-10°; developement, ascending.
Other notes are as Fig. 1.

***n*-Butanol-acetic acid.**—In this case, on spraying of an alcoholic solution of B. P. B. two acidic areas were revealed on a paper of the chromatogram, one was shown extended from one end of the strip to $R_F$ value of about 0.2, the other ahead of the former, as shown in Fig. 3 a (the former was not represented in the figure to avoid confusion). As the size of the former is independent of the concentration of hydrochloric acid, it is inferred that this area does not indicate the presence of hydrochloric acid but that of remaining acetic acid. On the contrary, as the size of the latter area grows larger with an increase in the concentration of hydrochloric acid, obviously this area shows the diffused one of hydrochloric acid. The latter acid was found to increase slightly the $R_F$ values of amino acids which were located near its field, namely alanine, glycine and aspartic acid (see Fig. 3 a). On the other hand the $R_F$ value of arginine was rather decreased, the results of which observed in various concentrations of hydrochloric acid were separately given below, to avoid confusion in the figure. Leucine, located far from the acidic area, was scarcely affected.

| (Conc. of HCl): | (0 *N*) | (0.6 *N*) | (1 *N*) | (2 *N*) | (6 *N*) | (12 *N*) |
|---|---|---|---|---|---|---|
| Arginine: | 0.19 | 0.18 | 0.17 | 0.16 | 0.16 | 0.16 |

By supplementary experiments, it was also proved that the effect on proline and glutamic acid was similar to that on alanine. Above

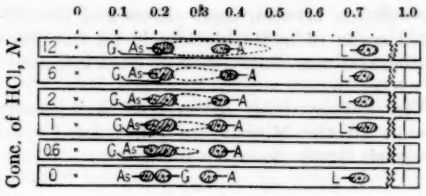


Fig. 3a

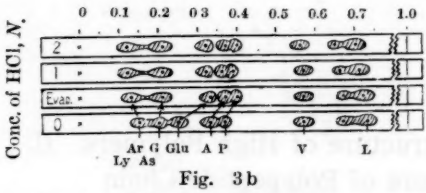


Fig. 3b

Figs. 3a and 3b.—Chromatograms of amino acids in hydrochloric acid of various concentrations developed by *n*-butanol-acetic acid. Temperature, *ca.* 10°; development, descending; P, proline, Glu; glutamic acid; Ly, lysine. Other notes as Fig. 1.

all, the effect on glutamic acid was the most remarkable. These were shown in Fig. 3 b, where 10% solutions of the dried digestion product of casein with trypsin in 2 *N* and 1 *N* hydrochloric acid or merely in distilled water respectively, were used for chromatography as usual. On the one hand, the solution of the above product in 6 *N* hydrochloric acid was also prepared and evaporated to dryness on steam-bath repeatedly four times to remove hydrochloric acid and chromatographed, the result of which was shown in the third strip of the figure. One may notice that a similar effect also appears on it as that of 2 *N* or 1 *N* acid.

**Phenol.**—When phenol was used as a solvent the effect of hydrochloric acid hardly appeared on the chromatograms of amino acids of higher $R_F$ values, namely leucine, valine, alanine and arginine, as shown in Fig. 4.

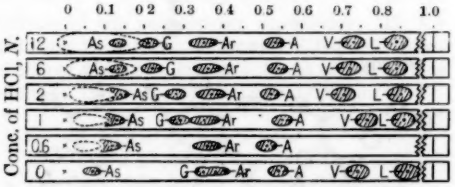


Fig. 4.—Chromatograms of amino acids in hydrochloric acid of various concentrations developed by phenol. Temperature, *ca.* 20°; development, ascending.
Other notes are as Fig. 1.

However, as the diffused area of hydrochloric acid was here revealed near the original point on the strip by B. P. B. (see Fig. 4), the amino acids of comparatively lower $R_F$ values and hence located about this area, namely glycine and aspartic acid, were affected by hydrochloric acid obviously as expected; the former decreased, while the latter increased.

**Collidine-lutidine.**—On developing with collidine-lutidine, a yellow separate band, perhaps connecting with the hydrochloric acid,[10] usually appeared just ahead (at *ca.* 0.65 in $R_F$) of the location of leucine as shown in Fig. 5, by revealing with B. P. B. The shape of

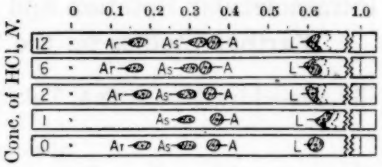


Fig. 5.—Chromatograms of amino acids in hydrochloric acid of various concentrations developed by collidine-lutidine. Temperature, 20°; development, ascending.
Other notes as Fig. 1.

leucine was distorted by this band, but its $R_F$ value and coloring hardly varied in any concentration of hydrochloric acid. As to the other amino acids, almost none of the effect of hydrochloric acid was found on the $R_F$ value, shape and coloring of the chromatogram (see Fig. 5). Among them, glycine was omitted from the figure to avoid confusion, and its $R_F$ values observed in various concentrations of hydrochloric acid were given below:

| (Conc. of HCl): | (0 *N*) | (1 *N*) | (2 *N*) | (6 *N*) | (12 *N*) |
|---|---|---|---|---|---|
| Glycine: | 0.32 | 0.34 | 0.33 | 0.33 | 0.35 |

Therefore, it is the most desirable way to use collidine-lutidine as the solvent in case some hydrochloric acid may be contained in the sample to be analyzed by paper chromtography.

## Summary

To explain what effect hydrochloric acid, contained in the sample to be analyzed, gives upon the paper chromatograms of amino acids, $R_F$ values of some amino acids dissolved in hydrochloric acid of various concentrations were observed with different kinds of solvent.

(10) Probably the hydrochloric acid composes a salt with collidine-lutidine.

In case of *n*-butanol, hydrochloric acid was found to increase the $R_F$ value of each amino acid with an increase in the concentration of hydrochloric acid, while in isovalerianic acid, on the contrary, to decrease that. On the one hand, only the amino acids of lower $R_F$ values were affected by hydrochloric acid in the case of phenol. In butanol-acetic acid, on the other hand, some effect was also found, but in collidine-lutidine it was negligible. In all cases, the relation between these effects and the diffused areas of hydrochloric acid on the chromatograms was observed with great interest.

The authors wish to express their hearty thanks to the Ministry of Education for the Research Grant given for this work.

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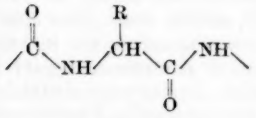
# Intramolecular Rotation and the Structure of High Polymers. II. Further Studies on the Structure of Polypeptide Chain

By San-ichiro MIZUSHIMA, Takehiko SHIMANOUCHI and Masamichi TSUBOI

(Received June 19, 1950)

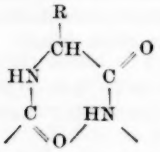
## I. Proposed Configurations of a Polypeptide Chain

Based on the energy consideration of intramolecular rotation about a single bond as axis, two of us proposed two unit structures of a polypeptide chain,[1]




(E-form)

and




(B form).

Taking into account that all amino acid residues of the polypeptide chain are in L-forms, we can construct various configurations of a polypeptide chain shown in Fig. 1, of which EEE$\cdots$ corresponds to the extended form presented by Meyer and Mark in 1928,[2] BBB$\cdots$ to a possible configuration of $\alpha$-keratin presented by us in 1947,[1] and independently by Ambrose *et al.* in 1949,[3] and BBEBBE$\cdots$ to another possible configuration of $\alpha$-keratin presented by us in 1948.[1] These figures represent

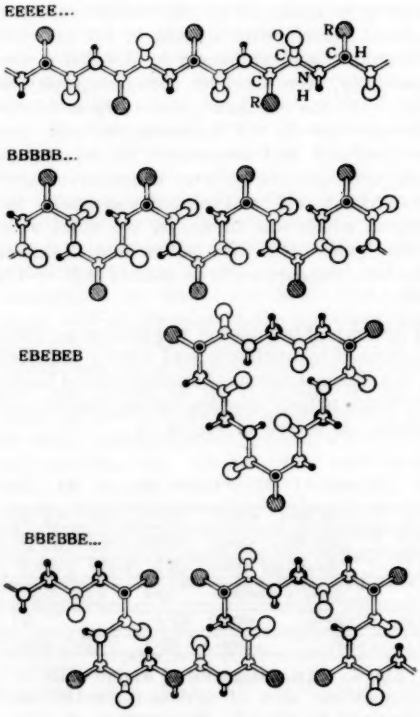



Fig. 1a.—Various configurations of polypeptide chain.

(1) Shimanouchi and Mizushima, *Kagaku*, **17**, 24 and 52 (1947); *Bull. Chem. Soc. Japan*, **21**, 1 (1948); *Chem. Abst.*, **43**, 8843 (1949).

(2) Meyer and Mark, *Ber.*, **61**, 1932 (1928).

(3) Ambrose, Elliot and Temple, *Nature*, **163**, 859 (1949).

the projections of the molecular configurations on a plane perpendicular to one of the carbon bonds, where for the sake of simplicity all the bond angles of carbon and nitrogen are assumed to be tetrahedral, all the bond lengths of C-C and C-N to be equal and all the intramolecular rotational states about the C-C and C-N bonds as axes to be either the *trans* ($\theta=0°$) or the *gauche* ($\theta=120°$).

and the intramolecular rotational angles to have the following values:

$$\theta(\text{NH-CO-C}_\alpha\text{-NH}) = 140°$$
$$\theta(\text{CO-C}_\alpha\text{-NH-CO}) = 110°,$$

the period along the chain is calculated as 5.14 Å. in agreement with the observed value 5.15 Å. and the N-H····O distance in the B

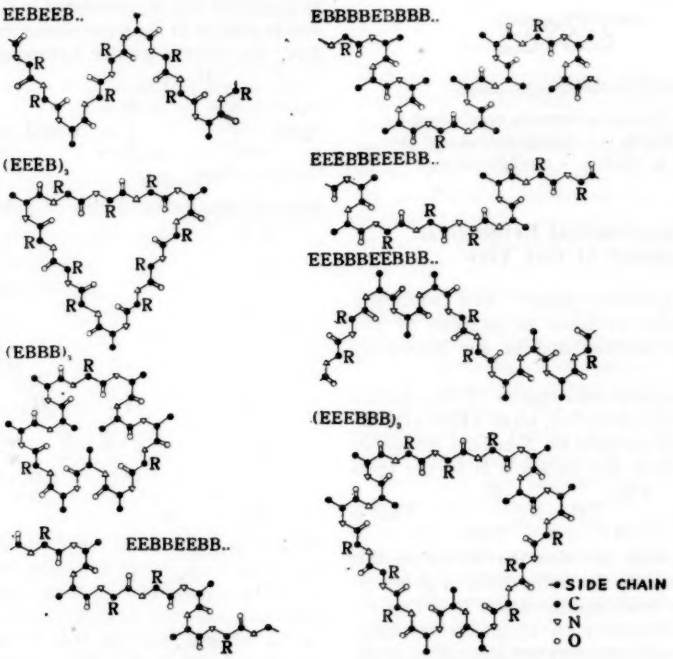



Fig. 1b.—Various configurations of polypeptide chain. (Side chains R perpendicular to the plane of the paper are not shown in the figures.)

In an actual case the bond angles and the bond lengths would be in some measure different from these ideal values and, moreover, the intramolecular rotational state would easily be changed to a certain extent under the influence of the neighboring molecules. If, for example, we assume the bond lengths and bond angles of polypeptide chain to have the values equal to those found for glycylglycine:[4]

| | |
|---|---|
| $C_\alpha-N = 1.48$ Å. | $\angle C-C_\alpha-N = 110°$ |
| $N-C = 1.29$ Å. | $\angle C_\alpha-N-C = 122°$ |
| $C-C_\alpha = 1.53$ Å. | $\angle N-C-O = 125°$ |
| $C-O = 1.23$ Å. | $\angle N-C-C_\alpha = 114°$ |

form as 2.52 Å. which is also reasonable.

As stated in our previous paper and as pointed out by Astbury, the presence of a twofold screw axis seems at first sight to be inconsistent with the fact that the 5.1 Å. meridional reflexion found in $\alpha$-keratin is strong and well defined.[5] However, according to the experimental result obtained by Bamford, Hanby and Happey, a kind of synthesized polypeptides gives an $\alpha$-keratin diagram in which the 5.28 Å. polar arc is weak and diffuse.[6] In view of this fact it is not unreasonale to propose the BBB···· configuration

(4) Hughes and Moore, *J. Am. Chem. Soc.*, **71**, 2618 (1949).

(5) Astbury, *Nature*, **164**, 439 (1949).

(6) Bamford, Hanby and Happey, *Nature*, **164**, 138 (1949).

as one of the possible structure of $\alpha$-keratin

In the case of BBEBBE···· configuration of $\alpha$-keratin the reflexion data can be explained more simply. If the mechanism of stretching of keratin fiber is explained in terms of the transformation of BBEBBE···· into EEE ····, the amount of elongation is calculated as about 90%, while the side chain spacing remains unchanged. (See Fig.2.)

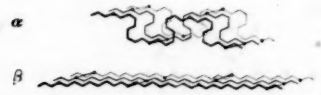
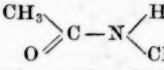


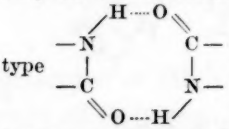

Fig. 2.—Natural $\alpha$-keratin considered to be in BBEBBE····· configuration and the $\beta$-keratin in EEE····· configuration.

## II. Experimental Evidence in Support of Our View

Since our previous paper[1] was published, the experimental evidence in support of our view has been accumulated in our laboratory as follows:

(1) Our experimental results of the Raman effect, infra-red absorption, ultra-violet absorption and dipole moment on N-methyl acetamide have shown that the molecule is in the *trans* configuration  .[7] This is in conformity with our view that all the peptide bonds contained in the main chain of polypeptide are in the *trans* configuration (see Fig. 1).

(2) When involved in hydrogen bonding, N-methyl acetamide shows two absorption peaks at 3.0 $\mu$ and at 3.2 $\mu$ arising from the N-H vibration.[7,8] N-ethyl acetamide and N-cyclohexyl acetamide, whose peptide bonds are shown to be in the *trans* configuration, also show two absorption peaks at the same wave lengths.[9] On the other hand $\delta$-valerolactam, $\varepsilon$-caprolactam, diketopiperazine, etc. whose peptide bonds are considered to be nearly in the *cis* configuration, show two absorption peaks at 3.1 $\mu$ and at 3.2 $\mu$.[8] This difference in wave length of the hydrogen-bonded N-H bands is attributable to the difference in the position of H-atom relative to other atoms and we can tell whether the peptide bond is in the *trans* configuration or in the *cis* configration according as the absorption peaks of the hydrogen-bonded N-H bands appear at 3.0 $\mu$ and 3.2 $\mu$ or at 3.1 $\mu$ and 3.2 $\mu$.

Since in egg albumin, horse serum albumin, etc. measured in our laboratory[8] and other proteins measured by Buswell *et al.*[10] only the 3.0 $\mu$ and 3.2 $\mu$ absorption peaks are observed except in salmin which shows the 3.1 $\mu$ peak arising from the side chain, the main chain of polypeptide can be considered to contain peptide bonds always in the *trans* configuration. Therefore, the intermolecular hydrogen bond of the type  would not be formed between two main chains of polypeptide.

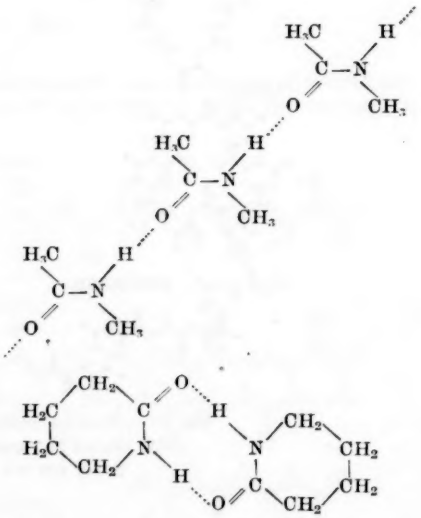


Fig. 3.—Configurations of associated molecules of N-methylacetamide and $\delta$-valerolactam.

(3) It has been shown by our infra-red absorption measurement that all the compounds except the first two shown in Table 1 can take the B form,[11,12] e.g. acetylglycine N-methyl amide can have a configuration:

(7) Mizushima, Shimanouchi, Nagakura, Kuratani, Tsuboi, Baba and Fujioka, *J. Am. Chem. Soc.*, **72**, 3490 (1950)

(8) Tsuboi, *Bull. Chem. Soc. Japan*, **22**, 215 and 255 (1949).

(9) Buswell, Rodebush and Roy, *J. Am. Chem. Soc.*, **60**, 2444 (1938).

(10) Buswell, Krebs and Rodebush, *J. Phys. Chem.*, **44**, 1126 (1940).

(11) Mizushima, Shimanouchi, Tsuboi, Sugita and Kato, *Nature*, **164**, 918 (1949).

(12) Mizushima, Shimanouchi, Tsuboi, Sugita, Kato and Kondo, to be published shortly.

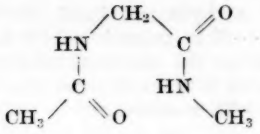


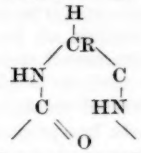
Table 1

Absorption Peaks Arising from N-H Vibrations

| Compound | Absorption peaks arising from the vibration of N-H group in the free state, $\mu$ | Absorption peaks arising from the vibration of N-H group involved in intramolecular hydrogen bonding, $\mu$ |
|---|---|---|
| $CH_3CONHC_6H_5$ | 2.90 | 2.97 (intermolecular) |
| $CH_3CONHCH_3$ | 2.88 | 2.97 (intermolecular) |
| $CH_3CONHCH_2CONHC_6H_5$ | 2.90 | 3.00 |
| $CH_3CONHCH_2CONHCH_3$ | 2.90 | 2.98 |
| $CH_3CONHCHCONHC_6H_5$ (CH$_2$, CH, $CH_3$, $CH_3$) | 2.90 | 3.00 |
| $CH_3CONHCHCONHCH_3$ (CH$_2$, CH, $CH_3$, $CH_3$) | 2.90 | 2.98 |

In very dilute solutions these substances show two absorption peaks as shown in Table 1, of which the 2.9 $\mu$ peak can be assigned to the vibration of N-H group in the free state and the 3.0 $\mu$ peak to that involved in the *intramolecular* hydrogen bonding. The soundness of the assignment of the 3.0 $\mu$ peak to the *intramolecular* hydrogen bonding and not to the *intermolecular* hydrogen bonding can be seen from the fact that the intensity ratio of the 3.0 $\mu$ to the 2.9 $\mu$ band is independent of the concentration in dilute solutions of acetylglycine N-methyl amide while in solutions of acetanilide and N-methyl acetamide for which only the *intermolecular* hydrogen bonds are formed, the 3.0 $\mu$ band becomes weaker with increasing dilution until it disappears practically. (See Table 1.) Thus there is no doubt that molecules of the substances shown in Table 1 can take the B form. This would also be an evidence for the existence of the B form for certain amino acid residues contained in the polypeptide chain.

## III. Configurations Presented by Other Investigators

Let us next discuss on the molecular configurations presented recently by other investigators. The configuration of $\alpha$-keratin proposed by Huggins has six membered rings similar to that of our configuration BBB$\cdots$, but it is different from ours with respect to the position of the side chain.[13] In the unit structure of his model  H and R are interchanged when compared with our B form. According to the energy consideration of intramolecular rotation his model containing more *gauche* forms than ours would be less stable than ours. This would be seen, for example, from the molecular configuration of monochlorocyclohexane recently determined by Kōjima.[14] Of the two conceivable configurations (equatorial and polar) of this molecule only the former has been observed in the Raman effect. This stable equatorial form corresponds in the present case to our B form and the unobserved polar form containing more *gauche* configurations than the equatorial corresponds to that presented by Huggins. The shape of pyranose rings determined by Reeves would provide us with another evidence in support of this view.[15]

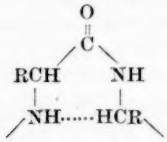
Based on the measurement with polarized infra-red radiation Ambrose *et al.* proposed independently a molecular model of $\alpha$-keratin identical with our model BBB$\cdots$.[3] This would be another evidence in support of our view. However, their experimental result can also be explained with our model BBEBBE$\cdots$. More detailed discussion on the observed dichroic ratio and the real molecular configuration stated in section I would be desirable.

Darmond and Sutherland measured the N-H frequencies of compounds containing peptide bonds, but arrived at a conclusion different from ours.[16] They consider that the 3.0$\sim$3.1 $\mu$ band arises from a configuration different from that to which they assigned the 3.2 $\mu$ band. According to our measurement, however, both of the 3.0 $\mu$(or 3.1 $\mu$) and the 3.2 $\mu$ bands arise from a single configuration in the case of N-methyl acetamide, acetanilide, and diketopiperazine in the solid state and $\delta$-valerolactam and $\varepsilon$-caprolactam in solutions.[7,8] (The proof for the single configuration for these substances is given by the X-ray diffraction, Raman effect,

(13) Huggins, *Chem. Rev.*, **32**, 195 (1943).
(14) Kojima, Private Communication.
(15) Reeves, *J. Am. Chem. Soc.*, **72**, 1499 (1950).
(16) Darmond and Sutherland, *Nature*, **164**, 440 (1949).

infra-red absorption and dielectric measurement). For the dimer of carboxylic acids it is also known that more than one O—H vibration arise from a single configuration.[17] Therefore, the appearance of more than one N—H stretching frequencies cannot be a proof for the existence of more than one type of hydrogen bond. Thus the objection of Darmond and Sutherland to the structure of $\alpha$-keratin proposed by us and by Ambrose *et al.* cannot be approved by our infra-red measurement.

Quite recently Kanda has presented another configuration of $\alpha$-keratin, the unit structure of which has the following form:[18]



His discussion is based on the consideration of interatomic distances, but not of the energy relation of the intramolecular rotation. According to the latter point of view, such a configuration is not acceptable and moreover, the hydrogen bond CH$\cdots$N contained in his configuration is supported by no experimental evidence. In the case of N$\equiv$C—H there is evidently a hydrogen bond of the type N$\equiv$CH$\cdots$N as pointed out by Kanda, but this is due to the abnormal property of the CH group of N$\equiv$C—H given rise to by the resonance with a structure

$$N^- = C^+ - H.$$

On the contrary CH groups contained in the main chain of polypeptide —CO—CHR—NH— have normal property and the nitrogen atoms next to carbon atoms have rather acidic property. Therefore, a resonance structure such as $>C^+$—H cannot be considered and the nitrogen atoms are not qualified to be a proton accepter. It has never been reported that the hydrogen bond of the type C—H$\cdots$N is formed between such a CH group and a nitrogen atom.

Kanda's objection to our configuration of $\alpha$-keratin is based on the consideration of the interatomic distances for the ideal model shown in Fig. 1. However, as stated in Section I, a reasonable change of bond distances, bond angles and intramolecular rotational states from the ideal values reconciles our configuration with the experimental data.

In conclusion we wish to thank Drs. N. Tanaka and K. Kuratani for their discussions. This work was aided by a grant from the Ministry of Education.

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(17) Buswell, Rodebush and Roy, *J. Am. Chem. Soc.*, **60**, 2239 (1938); Wall and Claussen, *J. Am. Chem. Soc.* **61**, 2812 (1939).

(18) Kanda, *Report of the Annual Meeting of the Chemical Society of Japan*, (1950).

# On the Retardation of Catalysis. The Catalytic Reaction between Acetylene and Hydrogen

By Kenzi TAMARU

(Received June 20, 1950)

## Introduction

Generally speaking, when acetylene and hydrogen react on a hydrogenating catalyst, there occur three kinds of reaction simultaneously, that is, hydrogenation of acetylene to ethylene and, further, to ethane, and hydrogenation-polymerization of acetylene.

In the case of palladium-alumina (1 : 100) catalyst, as shown in the preceding paper,[1] the hydrogenation of acetylene proceeds in two distinct steps at room temperature. The first step consists of the reduction and polymerization of acetylene and the second step the reduction of ethylene to ethane. The second

(1) K. Tamaru, *Bull. Chem. Soc., Japan*, **23**. 64 (1950).

reaction starts when all acetylene has been consumed, accompanying a sudden increase in the rate of hydrogenation. This phenomenon shows that acetylene is so strongly adsorbed on the surface of the catalyst that ethylene is not to be reduced till all acetylene has been used up. Consequently, it was found that the final products of the hydrogenation can be wholly made to ethylene or ethane by taking the proper composition of the reactants at the beginning.

As polymers, the polymerization products of acetylene, retard the hydrogenation reaction, the rates of hydrogenation should be corrected for this retardation in the kinetical treatment. In this study, experiments were carried out on the retardation of both steps of the hydrogenation by polymers as well as by some salts. And some considerations were given for the property of the "active region" on the catalyst surface.

## Experimental

Hydrogenation of acetylene on the palladium-alumina (1:100) catalyst was investigated by the static method. The preparation of the catalyst and the experimental procedure were the same as those in the preceding paper.[1]

The initial hydrogenation velocity of the first step was strictly proportional to the amount of the catalyst, but as the reaction proceeded the polymers that deposited on the surface of the catalyst retarded the reaction with degrees varying according to the amount of the catalyst and thus no proportionality was seen between the amount of the catalyst and the reaction rate. From the same reason the second step of the reaction had also no proportionality between them.

Thus, being able to calculate the amount of the deposited polymers from the gas analysis and the pressure decrease as shown in the previous paper,[1] the relation between the hydrogenation rate of ethylene ($k_2$) and the amount of the polymers were obtained by varying the amount of the catalyst or the composition of the reactant. This is shown in Fig. 1, where the amount of the deposited polymers were represented by the volume of acetylene in abscissa. The retardation curve of the first step ($k_1$) was obtained in the following ways. In the first method, $k_1$ was obtained from the reaction velocity at the last moment of the step, that is just before all acetylene had been used up, and the dependence of $k_1$ upon the amount of the deposited polymers was found. In the second method, the catalyst was used repeatedly and the progressive retardation caused by the accumulation of the polymers was observed in both steps. The results are shown in Fig. 2, where

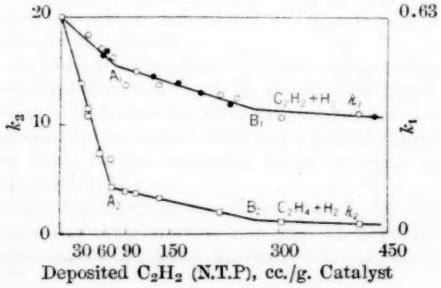


Fig. 1.—Retardation by deposited polymers at 30°C. (Catalyst: Pd-Alumina (1:100)).

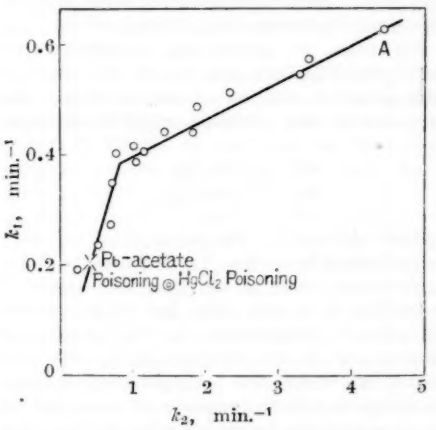


Fig. 2.—Progressive retardation by polymers and poisoning by salts.

$k_1$ in the ordinate was obtained from the initial velocity of the first step and $k_2$ in the abscissa was the velocity constant of the following second step calculated without any correction for the retardation of the polymers. Combining this with $k_2$ curve in Fig. 1, the dependence of $k_1$ upon the amount of the deposited polymers could also be obtained. Both results coincide with each other as shown in Fig. 1, where the results by the first method is represented by the white circles and the other by the black circles. The value of $k_2$ at no retardation was obtained from the reaction between ethylene and hydrogen.

It is shown in Fig. 1 that the retardation curves of $k_1$ and $k_2$, as it were, consist of three linear parts up to 450 cc. of acetylene deposited per gram catalyst and the two reflection points are designated by $A_1$, $B_1$ and $A_2$, $B_2$, respectively.

The hydrogenation rates were affected by washing the catalyst with solutions of some salts. The results of the poisonings by lead acetate and mercuric chloride solutions of which concentrations were 1.0% and 0.003%, respectively, were shown in Fig. 2, where the experimental conditions were the same as those used in the experiments of the progressive retardation mentioned above.

## Consideration

Without correcting the retardation of the polymers the hydrogenation rate of acetylene was roughly expressed by the following equation as shown in the preceding paper,

$$-\frac{dP}{dt} = \frac{k_1 P_{H_2}}{1 + b P_{C_2H_2}} \qquad (1)$$

where $P$ denotes the pressure of the gas in mm.-Hg, $t$, time in minute, $b$ and $k_1$ the constants. This equation was analogous to that given by Cremer and co-workers.[2]

But when we correct the retardation referring to Fig. 1, it was found that the net hydrogenation velocity of acetylene is to be expressed by the following empirical formula,

$$-\frac{dP}{dt} = \frac{k_1 P_{H_2}}{1 + b\sqrt{P_{C_2H_2}}} \qquad (2)$$

instead of Eq. (1). The value of $k_1$ at 30°, as calculated from Eq. (2), is shown in Table 1, the mean value of which is 0.63, while $b$ is 0.0774. It is seen that the hydrogenation velocity is independent on the pressure of ethylene. $k_1$ is also obtained to be 0.63 from Fig. 3, in which the hydrogenation velocity of acetylene and the pressure of hydrogen at the moment just before all acetylene has been used up are plotted.

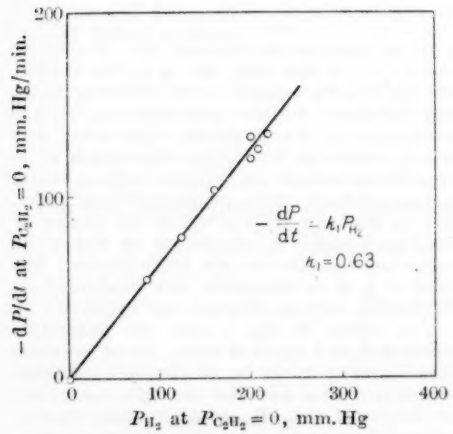


Fig. 3.—A relation between the reaction velocity and hydrogen pressure at the last moment of the acetylene hydrogenation.

All the deposited polymers are not necessarily to be considered to cover the surface of palladium, as they are also possibly adsorbed by the carrier. Consequently, the reflections of $k_1$ and $k_2$ curves in Fig. 1 may be attributed to the sudden change in the deposition of the polymers on the palladium surface, for example, when they saturate the palladium surface. It may also be attributed to another reason, for instance, to the discontinuous change in the fraction of the "active regions" on the catalyst surface by the preferential deposition of the polymers.

Table 1

Velocity Constant of the Hydrogenation of Acetylene at 30°C.

| $C_2H_2$, mm. Hg | $H_2$, mm. Hg | Acetylene Content, % | $k_1$, min.$^{-1}$ |
|---|---|---|---|
| 78 | 404 | 16.2 | 0.60 |
| 66 | 193 | 25.4 | 0.67 |
| 120 | 350 | 25.5 | 0.63 |
| 182 | 332 | 35.4 | 0.64 |
| 70 | 124 | 36.3 | 0.68 |
| 104 | 189 | 35.6 | 0.61 |
| 187 | 288 | 39.4 | 0.59 |
| 235 | 250 | 48.5 | 0.65 |
| 263 | 207 | 55.9 | 0.69 |
| 270 | 167 | 61.8 | 0.68 |

But when we plot $k_1$ against the corresponding $k_2$ of equal retardation calculated from the velocity of the first step at the last moment of the step and the velocity constant of the following step, a reflection is observed at A, while not at B as shown in Fig. 4, where

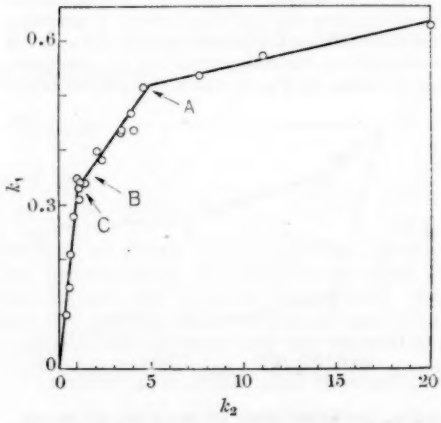


Fig. 4.—$k_1$–$k_2$ relation at equal retardation.

(2) E. Cremer, C. A. Knorr and H. Plieniger, *Z. Elektrochem.*, **47**, 737 (1941).

A corresponds to $A_1$ and $A_2$, and B to $B_1$ and $B_2$ in Fig. 1. Thus we shall see that the reflection at $A_1$ or $A_2$ in Fig. 1 is not caused by the discontinuous change in the deposition of the polymers on the surface of the palladium, but the discontinuous cnange in the fraction of the "active regions" on the catalyst surface itself. It is not the case, however, at $B_1$ or $B_2$, as it shows no reflection in Fig. 4.

According to the statistical mechanical treatment on the heterogeneous reaction by J. Horiuti,[3] the rate of the elementary reaction which proceeds in $G$ equivalent seats of critical complex is given by the following equation,

$$\overrightarrow{k} = \kappa \frac{kT}{h} G q \Theta_0 a_1$$

where $\Theta_0$ is the probability that the critical complex is absent and $a_1$ is the activity of the initial complex of the elementary reaction and $q$ is approximately equal to $e^{-\varepsilon/kT}$, where $\varepsilon$ is the activation energy of the reaction.

In the present case $\kappa$, $kT/h$, $G$, $a_1$, in the equation are assumed to be constant in each step, so that

$$\overrightarrow{k} = Ce^{-\varepsilon/kT}\Theta_0.$$

Now if we assume that the value of $\varepsilon$ is constant and the rate-determining step of the reaction does not change throughout the retardation, the reaction rates are only dependent on $\Theta_0$ and thus $k_1$ and $k_2$ are to be parallel.

In Fig. 4 three linear parts are observed and this may be associated with the three kinds of seat of critical complex or of "active region" on the catalyst surface. Accordingly, it seems to be possible to express from Fig. 4 that the ethane formation proceeds on the two kinds of the "active regions", while the hydrogenation of acetylene to ethylene on three kinds of them and that the first "active region" catalyzes the major part of the former, while only one fifth of the latter, and the third one about half of the latter and almost none of the former.

It is to be noted, however, that the preceding consideration is derived by the kinetical treatment of the reaction and, accordingly, is not necessarily associated with any physical meaning such as, for example, plane or edge of the catalyst.

The assumptions which are made for the preceding consideration may not all be available, especially when $\Theta_0$ approaches to zero, but it is likely to be considered to suggest that the two reactions of ethylene and ethane formation proceed on the various "active regions" with different ratios.

E. F. G. Herington[4] suggested that acetylene and ethylene are adsorbed on the various planes of nickel or cobalt with different ratios and that acetylene should be held on the long spacing of 3.50 Å., whereas ethylene on the short spacing of 2.47 Å. In the present experiment it is shown that the "active region," on which acetylene is not adsorbed, whereas ethylene hydrogenation can occur, is not present, as the inhibition of the ethane formation by acetylene is pronounced.

When the catalyst was washed by the solution of lead acetate or mercuric chloride, both rates of the hydrogenation were retarded as shown in Fig. 2. It was found that the former solution retards the ethane formation much more than the ethylene formation. T. Yamanaka[5] recently reported that palladium-kieselguhr catalyst treated by the lead acetate solution was good to apply to ethylene formation from acetylene by the flow method and this fact coincides with the result of the present experiment by the static method. As to the poisoning of mercuric chloride, $k_1$ and $k_2$ were approximately equally retarded. The results by the two salts suggest that the inorganic salts do not analogously poison the catalyst.

## Summary

1) The retardation of hydrogenation of acetylene on the palladium-alumina (1 : 100) catalyst by the deposited polymers and some inorganic salts were investigated.

2) By correcting the retardation of the polymers, which deposited on the surface of the catalyst during the reaction, it was found that the net hydrogenation rate of acetylene was expressed by the following equation,

$$-\frac{dP}{dt} = \frac{k_1 P_{H_2}}{1+b\sqrt{P_{C_2H_2}}}.$$

3) It was suggested that both catalytic hydrogenations of acetylene and ethylene proceeded on a few "active regions" of the catalyst with different ratios, and taking some assumptions, the rôle of the various "active regions" on the two hydrogenations was considered.

(3) J. Horiuti, *Bull. Chem. Soc. Japan*, **15**, 210 (1938).

(4) E. F. G. Herington, *Trans. Farad. Soc.*, **37**, 361 (1941).

(5) T. Yamanaka, *J. Phys. Chem. Res. Inst., Tokyo*, **25**, 329, 481 (1949).

4) It was shown that lead acetate solution poisoned the ethylene hydrogenation much more than the acetylene hydrogenation and this fact coincides with the result of the flow method.

The author expresses his sincere thanks to Prof. J. Sameshima for his kind guidance and encouragement throughout this work and to Dr. T. Kwan of Hokkaido University for his valuable suggestion.

The expense for the experiments has been defrayed from the Scientific Research Expenditure of the Department of Education.

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# Dispersity of Catalyst and Catalytic Activity. Catalytic Hydrogenation of Acetylene on the Pd-Kieselguhr Catalyst

Kenzi TAMARU

(Received July 17, 1950)

## Introduction

In general by the term "carrier" is meant an inert material which acts as a support for the catalyst.(1) A carrier may be used because it provides a larger contact surface and, at the same time, a more equal distribution of the catalyst. By preventing recrystallization, or baking together, it preserves a fine dispersion of the catalyst and hinders aggregation which otherwise would occur. A carrier may influence indirectly the activity of a catalyst by protecting it from poisons. Thus, many functions of a carrier are thought to vary with individual cases. But among these functions the case that a carrier may influence the properties of the catalyst proper is of interest.

Adadurow(2) suggested that a carrier is not at all an indifferent support for a catalyst, but that it produces a deforming action upon the atoms or molecules of the catalyst, changing their catalytic properties.

K. Yoshikawa(3) found that when acetylene is hydrogenated on a Pd-kieselguhr catalyst by the flow method, the yield of ethylene was much affected by the dispersity of palladium on the carrier, and that the catalyst with 500 ~ 1500 parts of kieselguhr being added to one part of palladium was well employed to get ethylene from acetylene. This markedly selective action of the catalysts produced by varying the dispersity of the catalytic agent on an inert substance, can not be explained from the standpoint of the problem of the surface area of the catalyst.

To explain this fact, Yoshikawa assumed the co-activation of neighbouring active centers on the catalyst surface by the heat of the reaction. He thought that the heat produced by the reaction on one active center activates the neighbouring hitherto inactive center, and the newly activated center excites in the same manner the next center as well as the original active point by further reaction and thus the proportion of stimulated active points increases and the reaction is correspondingly accelerated. If the dispersity of the catalytic agent is sufficiently large, the distance between the neighbouring active points may be long enough to disable the efficient co-activation, so that the catalytic centers can not display their full activities. There may be cases where the hydrogenation of acetylene to ethylene is possible but the hydrogenation to ethane is impossible.

Recently T. Yamanaka(4) and T. Takeshima,(5) having investigated the reaction on the Pd-kieselguhr catalyst by the flow method, discussed the mechanism the reaction on the basis of the modified coactivation theory.

The present author(6) previously investigated

(1) S. Bergman, J. C. Morrell and G. Egloff, "Catalysis" P. 426, New York (1940).

(2) Adadurow, *Zhur. Fiz. Khim.*, **3**, 489, 496 (1932); **6**, 206 (1935); *Zhur. Khim. Prom.*, **6**, 805 (1928).

(3) K. Yoshikawa, *Bull. Chem. Soc., Japan.*, **7**, 201 (1932).

(4) T. Yamanaka, *Sci. Rep. Sci. Res. Inst., Tokyo*, **25**, 329, 481 (1949).

(5) T. Takeshima, *ibid.*, **24**, 199, 303 (1948).

(6) K. Tamaru, this Bulletin, **23**, 28 (1950).

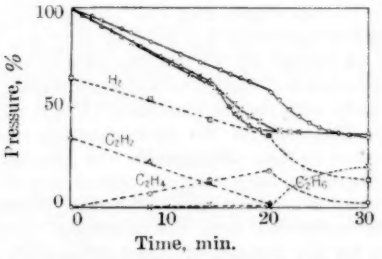
the hydrogenation of acetylene on Pd-alumina catalyst by the static method and found that the hydrogenation of acetylene proceeds in two distinct steps at room temperature. The first step consists of the reduction and polymerization of acetylene and the second step the reduction of ethylene to ethane. The second reaction starts after all acetylene is used up, accompanying a sudden increase in the rate of hydrogenation, and this fact was explained by the strong adsorption of acetylene on the surface of the catalyst proper.

In this study experiments were carried out on the Pd-kieselguhr catalyst of various dispersities and the rôle of the carrier or the problem of the dispersity of the catalyst was considered.

## Experimental

As in the previous experiments,[6],[7] Pd-kieselguhr catalyst was prepared from palladium chloride solution, into which kieselguhr[8] was added, by reduction to metal with formaldehyde. The dispersity of palladium on the kieselguhr was determined by the ratio of palladium to the carrier.

The experiments were carried out by the static method and their procedure was the same as that in the preceding papers.




—×—×—: Pd-Kieselguhr (1 : 750) Catalyst (0.600 g.) $C_2H_2$, 156 mm.Hg; $H_2$, 295 mm.Hg.
—⊙—⊙—: Pd-Kieselguhr (1 : 250) Catalyst (0.192 g.) $C_2H_2$, 155 mm.Hg; $H_2$, 288 mm.Hg.
—△—△—: Pd-Kieselguhr (1 : 100) Catalyst (0.098 g.) $C_2H_2$, 148 mm.Hg; $H_2$, 285 mm.Hg.

Fig. 1.—The experiments were carried out at 30° and the composition change of the reactant in the second experiment is shown by the broken lines.

The results of the experiments with the catalysts of various dispersities at 30° are shown in Fig. 1. The second step of the hydrogenation is retarded by the polymers deposited on the catalyst surface as discussed in the preceding paper[7] and, consequently, the velocity of the ethylene hydrogenation was obtained separately from the reaction between ethylene and hydrogen, the results of which are shown in Fig. 2, where the velocity constants are taken to the ordinate and the ratio of palladium to kieselguhr to the abscissa.

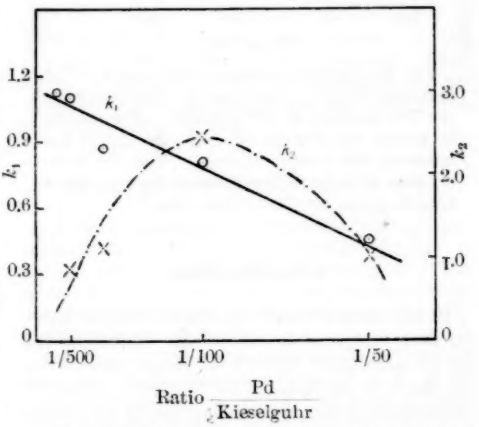



Fig. 2.—The relation between the catalytic activity (velocity constant) and the dispersity of catalyst in the two hydrogenation reactions ($k_1$ is the velocity constant in acetylene hydrogenation and $k_2$ in ethylene hydrogenation.)

It is shown in Fig. 2 that the second step of the hydrogenation or ethylene hydrogenation to ethane becomes slower relative to the hydrogenation rate of acetylene when the dispersity gets larger. This tendency is also observed in Fig. 1, in which no correction was made for the retardation of polymers.

As the reaction temperature was raised, the second step became slower as compared to the first step, because the activation energy of the former was smaller than that of the latter, and the catalyst becoming less, the amount of the polymers deposited on the unit area of the catlyst surface was increased, thus retarding the former more extensively than the latter as shown in the preceding paper.[7]

The results of the experiments at various temperatures by Pd-kieselguhr (1 : 750) catalyst are shown in Fig. 3, along with the change of the composition of the reacting gas. It is seen that the second step becomes slower than the first step at the transition of the first and the second steps in the experiment at 74.0°.

The reaction rates of the two steps at various temperatures were expressed by the Arrhenius' equation, respectively, and the activation energy of the reaction was 10 and 8 kcal. in the first and the second steps, respectively.

(7) K. Tamaru, *ibid.* **23**, 180 (1950)
(8) For this sample commercial kieselguhr was purified by boiling with hydrochloric acid.

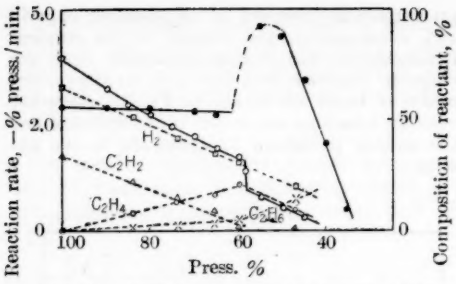



Fig. 3.—Reaction rates in the course of the hydrogenation of acetylene by Pd-Kieselguhr (1 : 750) catalyst at 30° and 74°. (The former is shown by the curve —●—●—, and the latter by the curve —○—○—, and the composition change of the reactant in the latter case is given by the broken lines.)

## Consideration

It had been found,[3] as mentioned previously, that acetylene is selectively hydrogenated by Pd-kieselguhr catalyst of larger dispersities by the flow method. This fact was explained by the assumption of co-activation of the neighbouring active centers on the catalyst surface, and it was assumed in this case that the active centers, not displaying their full activities, proceed the hydrogenation of acetylene to ethylene, but not of ethylene to ethane.

But present experiments showed all the catalysts of various dispersities to catalyze the hydrogenation of ethylene as well as acetylene and that in two distinct steps quite similarly with the Pd-alumina catalyst.

We see in Fig. 2 that the hydrogenation rate of the first step increases when the dispersity grows larger and that the ratio of $k_1$ to $k_2$ differs according to the dispersity of the catalyst.

If the carrier provides only a larger effective surface of the catalyst proper, the ratio should not differ by the dispersity, though the velocity constants of $k_1$ and $k_2$ may increase with the dispersity. This fact suggests that the dispersity contributes to the property, as well as to the area, of the catalyst surface.

In the preceding paper[7] it was suggested that the hydrogenations of acetylene and ethylene proceed on the various "active regions" with different ratios. Hence, the difference of dispersity is considered to have connection with the appearance of various "active regions" with different ratios, as the ratio of the two hydrogenation rates are differed by the dispersity.

For the practical purpose to obtain ethylene from acetylene by the flow method it was suggested in Fig. 1 and Fig. 2 that the catalysts of larger dispersities are good to apply, as the rate of ethane formation is slow relative to that of ethylene formation. This is supported by the above-mentioned results of Yoshikawa by the flow method.

The reaction rate of the second step became slower than that of the first step at the transition of the two steps as shown in Fig. 3. This inversion temperature was not characteristic to the catalyst proper itself, but was dependent on the dispersity of the catalyst.

The fact that the hydrogenations of acetylene and ethylene proceeded independently, obeying the Arrhenius' equation, and that the two reaction rates at the transition of the two steps inverted each other show that the adsorption of hydrogen on the catalyst surface or the supply of the reacting hydrogen is not rate-determining in the hydrogenation of ethylene, provided that the state of the reacting hydrogen is the same in both hydrogenations.

## Summary

1) Hydrogenation of acetylene on the Pd-kieselguhr catalyst of various dispersities was investigated.

2) It was shown that the hydrogenation of acetylene proceeded not only to ethylene, but also to ethane by all the catalysts of various dispersities, and that in two distinct steps quite similarly with the Pd-alumina (1:100) catalyst.

3) The ratio of the hydrogenation rate of acetylene to that of ethylene was affected by the dispersity, suggesting the appearance of the various "active regions" of the catalyst surface to be connected with the dispersity. It was large for the catalyst of larger dispersities and this fact explains the results previously obtained by the flow method.

4) Some considerations were given to the rate-determining step in the hydrogenation of ethylene.

The author expresses his sincere thanks to Prof. J. Sameshima and Dr. H. Akamatsu for their kind guidance and encouragement throughout this work. The expense for the experiments has been defrayed from the Scientific Research Expenditure of the Department of Eduction.

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# Application of Cation Exchange Resin to the Determination of Phosphorus in Foods

By Shohji KUBO and Chuichi TSUTSUMI

(Received July 19, 1950)

Cation exchange resin was first used by Herlich and Rieman[1] for the determination of phosphorus in phosphate rocks. They converted the hydrochloric acid solution of phosphate into a mixture of hydrochloric acid and phosphoric acid by cation exchange and titrated directly with 0.1 *N* alkali using comparison buffers. Dijksman[2] referred briefly to this method, and by the present authors the principle has been applied to the volumetric determination of phosphorus in foods[3] and the gravimetric adaptation of the exchange technique has been investigated.[4] However, it was found later that the volumetric method sometimes results in high values and that this was caused by the interference of carbonate in the concentrated alkali added before the titration. To avoid this difficulty in the present investigation the applicability of sodium regenerated cation exchange resin was examined as a substitute for the previously used hydrogen regenerated type and the following method is now recommended for the determination of phosphorus in foodstuffs.

## Reagents

Hydrochloric acid; 20%.

Standard sodium hydroxide solution; 0.05 *N*: carbonate free.

Sodium chloride solution; 5%.

Methyl red indicator; 0.1% alcoholic solution.

Phenolphthalein-thymol blue indicator; to 1 part of 0.1% solution of thymol blue in 50% alcohol add 3 parts of 0.1% alcoholic solution of phenolphthalein.

Acetate buffer, *p*H 4.63; mix 17 ml. of 1.3 moles sodium acetate, 25 ml. of 1.0 mole acetic acid and 360 ml. of water. To 50 ml. of this solution add 3 drops of methyl red.

Borate buffer, *p*H 8.98; mix 130 ml. of 0.1 mole borax, 40 ml. of 0.1 mole hydrochloric acid and 280 ml. of water. To 60 ml. of this solution add 3 drops of methyl red and 6 drops of the phenolphthalein-thymol blue indicator. Each buffer solution should be contained in an Erlenmeyer flask similar in size and color to the one used in the titration.

## Preparation of Exchange Column

Cation exchange resin; 100 to 200 mesh phenolmethylenesulfonic resin. Amberlite IR-100 or similar resins may be used. A resin synthetized in our laboratory was used in the present studies. Its total static exchange capacity was 2.28 milliequivalents per gram.

Six grams of the resin were suspended in the water and poured into an ordinary 25 ml. stopcock burette, the bottom of which had been plugged with absorbent cotton. Before each determination the exchanger was regenerated by pouring 100 ml. of sodium chloride solution through the column followed by 60 ml. of water in 3 portions. The rate of flow was adjusted by means of the stopcock to about 1 ml. per minute.

Hitherto, when using the exchange column, it was necessary to maintain the level of the solution several centimeters above that of the resin to prevent the formation of air bubbles between resin particles with the subsequent blocking of the column. However, in the case when the particle size is reduced to less than 100 mesh, the flowage stops automatically with the stopcock open when the solution reaches the bed level. The pressure drop caused by the small particle size did not obstruct the procedure, the maximum rate of flow being 3 ml. per minute under the experimental conditions. It is therefore necessary to regulate the rate of flow only once and thus the analyst is released from the continuous attention. For these reasons, the particle size of resin was reduced to 100-200 mesh. This made possible the simultaneous treatment of an increased number of samples without applying additional skill or the use of special apparatus.

## Procedure

Weigh 5 g. of sample (or less in the case of phosphate-rich materials such as rice bran) into a porcelain crucible or a small Pyrex beaker and ash at 550 to 600°. Dissolve the ash in hydrochloric acid and evaporate to dryness on a water bath. Add 0.5 ml. of 20% hydrochloric acid and *ca.* 5 ml. of water, heat on a water bath, filter,

(1) K. Herlich and W. Rieman, *Anal. Chem.*, **19**, 651 (1947).

(2) J. C. W. Dijksman, *Rec. trav. chim.*, **68**, 57 (1949).

(3) S. Kubo and C. Tsutsumi, *Report of the Food Research Institute (Tokyo)*, **3**, 72 (1950).

(4) *ibid.*, **3**, 83 (1950).

and wash with a small amount of water. Pass the filtrate and washings through the column at the rate of about 1 ml. per minute, collecting the filtrate in a 100 ml. Erlenmeyer flask. Wash with 25 ml. of water in 3 portions at the same rate and collect the washings in the same flask. The total volume will be about 50 ml. Add 3 drops of methyl red solution and adjust to *p*H 4.63 with 0.05 *N* sodium hydroxide solution using the acetate buffer. Usually less than 5 drops of the alkali will suffice for this purpose. Then add 6 drops of phenolphthalein-thymol blue indicator and titrate to *p*H 8.98 with 0.05 *N* alkali using the borate buffer. The titration is most preferably done under the light of a fluorescent lamp.

The phosphorus content of the sample is calculated from the volume of standard alkali required for the titration.

## Experiments and Discussion

As would be expected from the exchange equilibrium of the resin, any hydrochloric acid solution of phosphates, after passing through an adequate amount of sodium type resin, shows an almost constant *p*H regardless of the amount of free hydrochloric acid present, providing that it lies within a certain concentration range. In these experiments some salts were added to standard potassium acid phosphate solutions, and to these were added 1, 3, 5 and 10 ml. of *N*-hydrochloric acid respectively. After passage through a 5 g. column of sodium type resin, the solutions were made up to the volume, *p*H were measured, and the phosphoric acid contents determined by titration. The results are given in Table 1.

Table 1

*p*H and Phosphoric Acid in the Effluents

| | *N*-HCl added, ml. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | | 3 | | 5 | | 10 | |
| | *p*H | P[a] | *p*H | P | *p*H | P | *p*H | P |
| $KH_2PO_4$ | 4.9 | 4.13 | 4.7 | — | 3.9 | — | — | — |
| 〃 and Soln. A[b] | 4.0 | 4.13 | 4.0 | 4.10 | 3.8 | 4.10 | 1.3 | 4.11 |
| 〃 and soln. B[c] | 3.9 | 3.92 | 3.9 | 4.00 | 3.1 | 4.08 | 1.2 | 4.18 |

(*a*) ml. of 0.05 *N*-NaOH required for the titration. Theoretically expected value is 4.10.
(*b*) Solution A contains 10 mg. Ca and 6mg. Mg as calcium chloride and magnesium sulfate respectively.
(*c*) Solution B contains 12 mg. Mg, 9 mg. $Fe^{III}$ and 5 mg. Al as magnesium sulfate, iron alum and potassium alum respectively.

It is seen that when the amount of added hydrochloric acid is limited, the *p*H of the effluent always approximates to 4.0, and that the addition of only a few drops of 0.05 *N* alkali is necessary to adjust the *p*H 4.63. By eliminating the need for neutralization with concentrated alkali, as is required when the H-resin is employed, the method is simplified and carbonate interference is eliminated.

However, it is seen in Table 1 that when solution B is added, and at the same time, smaller amounts of hydrochloric acid are employed, the recovery of phosphorus is low even though cation exchange is complete. It is considered that the exchanged $Fe^{III}$ or $Al^{III}$ ion tends to adsorb some phosphoric acid, especially when the *p*H of the original solution is relatively high. The effects of the presence of ferric and ferrous iron and aluminum were studied, and from the results shown in Table 2, it is concluded that when smaller amounts of hydrochloric acid are used, only larger amounts of ferric iron will interfere with the accurate determination of phosphorus. Ferrous iron and aluminum showed no such tendency under the experimental conditions. However, in ordinary foodstuffs the contents of iron are not high enough to cause any difficulties. This can be said especially for cereals, beans, meat etc. where the iron levels are about the one hundredth that of phosphorus. Therefore, in practice, the interference of ferric iron can be

Table 2

Effects of Iron and Aluminum

(The figures represent the ml. of 0.05 *N*-NaOH required for titration. P present in each aliquot is 14.28 mg.)

| | Fe or Al added, mg. | *N*-HCl added, ml. 0 | 1 | 3 | 5 |
|---|---|---|---|---|---|
| $KH_2PO_4$ | — | 8.21 | 8.20 | 8.25 | 8.23 |
| $KH_2PO_4$ and $Fe^{II}$ | 1 | | 8.22 | 8.25 | 8.23 |
| (Mohr's salt) | 3 | | 8.21 | 8.23 | 8.25 |
| | 5 | | 8.25 | 8.24 | 8.29 |
| $KH_2PO_4$ and $Fe^{III}$ | 1 | | 8.24 | 8.25 | 8.26 |
| (iron alum) | 3 | | 7.99 | 8.17 | 8.22 |
| | 5 | | 7.71 | 8.04 | 8.19 |
| $KH_2PO_4$ and Al | 1 | | 8.24 | 8.23 | 8.22 |
| (potassium alum) | 3 | | 8.23 | 8.21 | 8.24 |

Table 3

Control Analysis

(The figures represent the ml. of 0.05 *N* NaOH required for titration.)

| | | *N*-HCl added, ml. 0 | 1 | 3 | 5 |
|---|---|---|---|---|---|
| Sample | P added | P found | | | |
| Rice bran | 8.10 | 8.07 | 8.12 | 8.10 | 8.14 |
| Soybean | 7.82 | 7.80 | 7.80 | 7.80 | 7.85 |

considered negligible, and this fact was confirmed by the following control analysis on soybean and rice bran.

A new method was adapted for the control analysis to prepare the sample solution. The sample (rice bran and soybean) was ashed, dissolved in hydrochloric acid, evaporated to dryness, redissolved, heated with dilute hydrochloric acid on a water-bath, and filtered to remove insoluble silica and carbon. The solution was passed through an enough amount of hydrogen regenerated cation exchanger to exchange all the cations in the solution. The resin was washed thoroughly with distilled water and then the adsorbed cations were effluated with 4% hydrochloric acid. The effluent was mixed with a known amount of phosphoric acid (nearly equivalent to the quantity in the original sample), evaporated to dryness, and redissolved in distilled water to a definite volume. By this means we obtained solutions which had the same mineral composition as the ash of the sample, their phoshorus contents being known.

Aliquots were taken and analysed by the above procedure and the values found were compared with the added amounts of phosphorus. As shown in Table 3, very satisfactory results were obtained on both rice bran and soybean.

From the above experiments and considerations, substitution of the sodium type exchanger for the hydrogen type is recommended, since it results in a more simplified and accurate method of determination.

The *p*H values used for the end points in the procedure were taken from the potentiometric titration data of Herlich and Rieman.[1] The phenolphthalein used by them was replaced by phenolphthalein-thymol blue indicator because of its greater sensitivity. By this means even the color change resulting from the addition of half a drop of 0.05 *N* alkali can be detected in the ordinary Erlenmeyer flask.

## Summary

A new volumetric determination of phosphorus in foods using a sodium type cation exchange resin was devised and critically investigated. The substitution of sodium for the hydrogen type exchanger eliminated errors resulting from the introduction of carbonate present in concentrated alkali. Moreover, the reduction of the resin particle size resulted in simplification of the procedure and reduced the skill necessary for its execution.

This method is accurate and applicable to the determination of phosphorus in ordinary foodstuffs, except when the sample contains unusually large amount of ferric iron. In such cases, the hydrogen type exchanger method[3] must be employed.

The authors give their sincere thanks to Taro Nagahara for his kind guidance throughout the cource of this study and to Dr. G. J. Mannering for his suggestions about the interferences of iron and aluminum.

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# On Some Applications of the Nakamura-Kuroda Theory of the Sedimentation of Colloid in a Vessel having Inclined Walls

By Tominosuke KATSURAI

(Received August 9, 1950)

The increase of sedimentation velocity of a colloid in an inclined test tube was first reported by Boycott.[1] This curious phenomenon drew attention of many workers, and gave rise to the publication of many papers containing tentative theories to explain the phenomenon. Among these works the joint work of Nakamura and Kuroda might be considered as representative.[2] They made it clear that the inclined wall lying above the colloid (Fig. 1 a) gives rise to the increase of

(1) A. E. Boycott, *Nature*, **104**, 532 (1920).

(2) H. Nakamura and K. Kuroda, *Keijo J. Med.*, **8**, 256 (1937). [Keijo = Seoul, Korea].

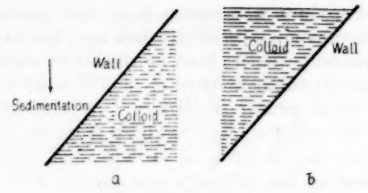



Fig. 1.

sedimentation velocity, while the wall situated below the colloid (Fig. 1 b) does not. From a very simplified assumption taking this fact into consideration, they gave a theory, though phenomenological in nature, which agrees with experiments quite well. Lately Kinosita examined the applicability of the Nakamura-Kuroda theory (abbreviated as N-K theory hereafter) in the settling of smokes and found that the theory holds as a whole.[3] In this paper, some results obtained by extending the fields of application of the N-K theory are given.

## Sedimentation of Colloid in a Tube consisting of Two Parts having Different Sectional Area

A simple illustration of the N-K theory can be found in the sedimentation of colloid in a vessel consisting of two cylinders having different radii (Fig. 2). We know that the essential point of the N-K theory is expressed as follows.

Fig. 2.

$h \times$ upper boundary area of suspended phase
$= s \times$ horizontal projection of the upper boundaries of the suspension, (1)

where $h$ = sedimentation velocity of colloid in a vessel having inclined walls,
$s$ = sedimentation velocity of colloid determined in a vertical cylinder of uniform section.[4]

Applying the relation (1) in this case, we have

$$hA_1 = sA_2$$

$$\text{or} \qquad h = s\,\frac{A_2}{A_1}, \qquad (2)$$

where $A_1$ and $A_2$ are the sectional areas of the upper and lower cylinders. This relation shows that the initial sedimentation velocity of a colloid increases in a vessel having the form of Fig. 2, and that the amount of increase is determined by the ratio of the sectional areas of the lower and upper parts.[5]

## Flotation of Emulsion in a Vessel under the Action of Oblique Centrifugal Force. Note on the Design of de Laval Centrifuge

It seems of interest to apply the N-K theory in a problem of centrifugal field. As an example, a problem of the design of de Laval centrifuge, which is usually used as cream separator, has been chosen. As is well known, the diagramatic sketch of the de Laval centrifuge is given in Fig. 3. Milk is kept in the spaces

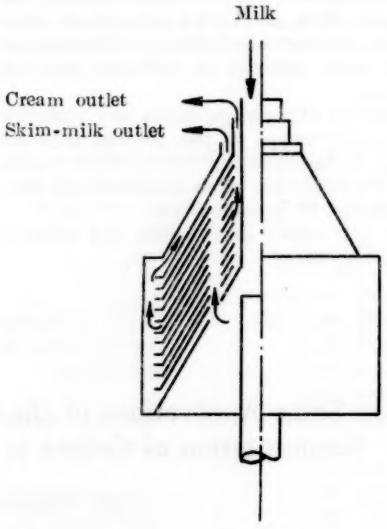



Fig. 3.

confined between two consecutive cones and is subjected to centrifugal force acting obliquely to the walls. In the actual case, the ratio between the slant height of cone and the

(3) K. Kinosita, *J. Colloid Sci.*, **4**, 525 (1949).
(4) *cf.* (3) p. 526 below.
(5) A test carried out by Mr. T. Matuhasi, using suspension of blood corpuscle in physiological saline solution, showed that this relation actually holds.

distance between cones is about 120 and the inclination (the angle between the direction of centrifugal force and the slant height) is about 45°. A cursory glance at literature fails to find any study stating how these values were chosen. We shall examine whether the N-K theory is of use in interpreting these values.

In order to apply the N-K theory we neglect the flow of milk and observe only the flotation of fat particles which takes place under the action of centrifugal force. We assume that milk is an emulsion which is characterized by a flotation constant $s_0$ and confine our attention to the behavior of the milk contained in the space ABA'D'CD between the two cones ABA' and DCD' (Fig. 4). $s_0$ is a constant

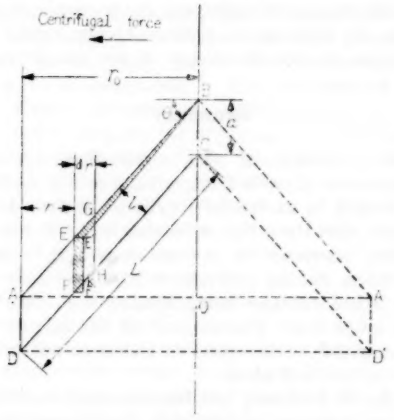



Fig. 4.

such that its product with centrifugal force gives the velocity of flotation. The conception of $s_0$ herewith is entirely in line with that of the sedimentation constant in ultracentrifuging.

Let us consider the milk in the parallelogram ABCD. The point A is taken as the origin of coordinate, and the distance from A toward the axis of rotation is taken as positive $r$. We denote the inclination by $\theta$, and the angular velocity by $\omega$. At first ABCD is uniformly filled with milk. With the elapse of time, flotation takes place and the boundary between skim-milk and cream moves toward the axis of rotation. We denote the boundary at the time $t$ by EF, the distance of EF from the bottom AD by $r$, and the position of EF after the time interval $dt$ by E'F'. From the definition of $s_0$, the distance EE' is given by $s_0\omega^2(r_0 - r)\,dt$. The particles along EB should also move toward the axis of rotation with the elapse of time. From the fact that centrifugal force is proportional to the distance from the axis of rotation, the particles situated along EB should be found along E'B after the elapse of time $dt$. Thus the boundary after the time interval $dt$ should be given by the line F'E'B. The small triangular area FF'K is neglected in the calculation. However, the actual boundary should remain always parallel to the axis of rotation. Following the principle of leveling action of the N-K theory, the position of the actual boundary GH is determined by the relation

$$\begin{aligned} &2\pi\,(r_0 - r)\,a\,dr \\ &= 2\pi\,(r_0 - r)\,a s_0\omega^2(r_0 - r)\,dt \\ &+\left[\frac{1}{3}\,\pi(r_0 - r)^2(r_0 - r)\tan\theta - \frac{1}{3}\,\pi\left\{r_0 - r - s_0\omega^2(r_0 - r)\,dt\right\}^2(r_0 - r)\tan\theta\right]. \end{aligned} \tag{3}$$

This represents the condition that the volume generated by the revolution of the hatched area EFF'E'B is equal to the volume generated by the revolution of the area EFHG around the axis BO. Neglecting the terms containing the square of $dt$, and rearranging the remaining terms, we get

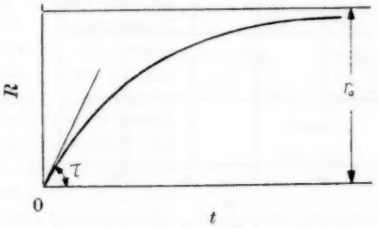
$$\frac{dr}{dt} = s_0\omega^2(r_0 - r)\left\{1 + \mu(r_0 - r)\right\},$$

where $$\mu = \frac{\tan\theta}{3a}.$$

Integrating and making use of the initial condition, $r = 0$ for $t = 0$, we get

$$r = r_0 - \frac{1}{\left(\mu + \frac{1}{r_0}\right)e^{s_0\omega^2 t} - \mu}. \tag{4}$$

This is the equation which gives the change of boundary with time. The graph of this equation is given in Fig. 5.




$$\tan\tau = s_0\omega^2 r_0\,(1 + r_0\mu)$$

Fig. 5.

As the measure of the efficiency of cream separation, we take the initial velocity of

flotation. We make $\left(\frac{dr}{dt}\right)_{t=0}$ from (4) and study its change with the inclination and the distance between the cones. By means of the relation $r_0 = L\cos\theta$, we get

$$\left(\frac{dr}{dt}\right)_{t=0} = s_0\omega^2 L\cdot\varphi(\alpha,\theta), \qquad (5)$$

where $$\varphi(\alpha,\theta) = \cos\theta(1+\alpha\sin 2\theta), \qquad (6)$$

$$\alpha = \frac{L}{6l}.$$

Thus we see that if $s_0$, $\omega$ and $L$ are given, the initial velocity is determined by the function $\varphi(\alpha,\theta)$. In Fig. 6 the values of $\varphi(\alpha,\theta)$ are plotted against $\theta$, taking $\alpha$ as parameter. The function $\varphi(\alpha,\theta)$ increases with the increase of $\alpha$. If we denote by $\theta_m$ the value of $\theta$ which makes $\varphi(\alpha,\theta)$ maximum, it increases with the increase of $\alpha$ at first suddenly and then approaches the limiting value 35°.(6) The relation between $\alpha$ and $\theta_m$ is given in Fig. 7.

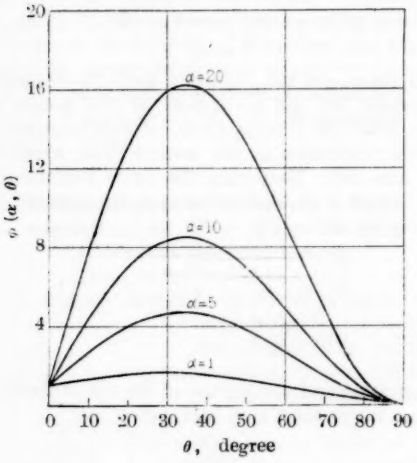



Fig. 6.

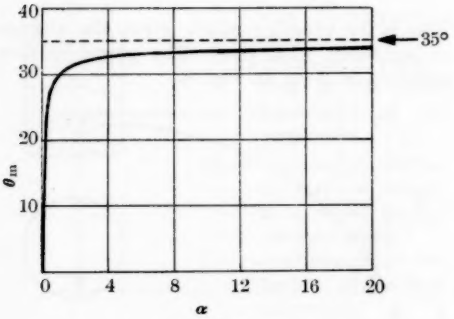



Fig. 7.

From the result obtained so far, larger value of $\alpha$ is desirable for higher efficiency. However, there is a limit for the value of $\alpha$ from the standpoint of mechanical construction, and we cannot increase it infinitely. The actual construction corresponds to the value 20 of $\alpha$. The value of $\theta_m$ corresponding to $\alpha$ greater than 10 is nearly 35°, while the actual inclination is 45°—50°. The coincidence is not very good. However, so long as there is no reasonable way of determining the proper inclination, the conclusion obtained hereby might be of some use for the design of the centrifuge.

## Summary

As a result of the application of the Nakamura-Kuroda theory of the sedimentation of colloid in an inclined cylinder it has been shown that the initial sedimentation velocity of colloid increases in a vessel consisting of two cylinders having different radii, the radius of the upper cylinder being smaller than that of the lower one. The amount of the increase is determined by the ratio of the sectional areas of the two cylinders.

The N-K theory has been formally extended in the flotation of emulsion in a centrifugal field, and the design of the de Laval centrifuge has been considered in the light of the results obtained. A feasible, though not quite rigorous, explanation of the consistency of the form of centrifuge has been given.

In conclusion, the author would like to express his cordial thanks to Professor S. Oka for his kind interest in this work.

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(6) This is the value of $\theta$ which makes $\varphi(\alpha,\theta)$ maximum when $\alpha$ is very great. It is obtained by solving $\frac{d\varphi}{d\theta} = 0$, neglecting the term which does not contain $\alpha$.

# Studies on the Gas Liquid Interface by Surface Potential Measurements. II. On the Solutions of Dicarboxylic Acids

By Toshizo ISEMURA and Hirosi HOTTA

(Received June 19, 1950)

Molecules of polar-nonpolar substances on the surface of a solution will probably take particular orientation at the gas liquid interface. The orientation of such molecules will be greatly affected with the molecular configuration of the adsorbed substances. Many investigations on the structure of monolayer of insoluble heteropolar compounds or the orientation of the molecules on water have been reported by many investigators.[1] The measurement of the surface potential of monolayer is one of the useful methods for studying the structure or the orientation of the monolayers. The method will also serve as the most useful one for the investigation on the nature of adsorbed surface layer of solutions. Nevertheless, relatively few investigations have already been reported.[2] Previously, one of the authors measured surface potential of aqueous solutions of some fatty acids.[3] Then, it was found that the surface potential of aqueous solutions of monocarboxylic acids such as *n*-butyric, *n*-valeric and *iso*-caproic acid reverses the sign of the potential in the region of concentration of high dilution. It was concluded that this reversal of the sign of surface potential is probably caused either by the dissociation of adsorbing acid or by the particular orientation of the adsorbed fatty acid molecules. In order to clarify the cause of the sign reversal of potential at high dilution and to obtain some informations of the molecular orientation of the adsorbed layer, we have measured in the present experiments the surface potential of aqueous and acidulated solutions of some dicarboxylic acids such as oxalic, fumaric, maleic, succinic, adipic, suberic, azelaic, sebacic and $\gamma\gamma'$-sulfonyl dibutyric acids. These acids, investigated in our present experiments, have polar groups at both ends of linear molecule and may lie probably flat in the adsorbed layer in virtue of two polar groups at the ends. $\gamma,\gamma'$-sulfonyl dibutyric acid has one more polar group at the midst of the molecule. Among the acids investigated, oxalic, maleic, fumaric and succinic acids always gave negative surface potential all over the concentration range. Other acids than these showed reversal of sign of the potential in the region of concentration of high dilution as in the cases of monocarboxylic acids. If the solution were acidulated by addition of small quantity of hydrochloric acid, the potential of the solutions was shifted to more positive values than the values of respective aqueous solutions. These results can readily be interpreted from the viewpoint that negative surface potential is principally caused from adsorbed fatty acid anions. At relatively high concentration, if the chain length of acid is sufficiently long, bending of the hydrocarbon chain of such dicarboxylic acid at the middle of a molecule would be caused by hydrophobic nature of hydrocarbon chain. Consequently, the adsorbed layer will much resemble to the adsorbed layer of monocarboxylic acid as indicated in Fig. 1. It was found that the expected resemblance of the surface layer of dicarboxylic acid solution to that of monocarboxylic acid was in part realized with concentrated solution of sebacic acid. One of the strong evidences which has rendered us to believe that the reversal of sign of potential is not caused by molecular orientation but mainly by dissociation of acid, is the following fact. The sign reversal of the surface potential was observed even with acidulated solution of $\gamma,\gamma'$-sulfonyl dibutyric acid which has one more polar group at the midst of molecule, so that the molecule can not bend anyway and must always lie flat.

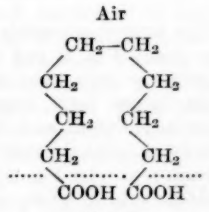

Fig. 1

(1) N. K. Adam, "The Physics and Chemistry of Surfaces," 72-77, Oxford, (1941).

(2) Bichat and Blondlot, *J. phys. Radium*, **2**, 548 (1883); Kenrick, *Z. physik. Chem.*, **19**, 625 (1896); Frumkin, *ibid.*, **109**, 34 (1924); **111**, 190 (1924); Sawai, *Trans. Faraday Soc.*, **31**, 764 (1935).

(3) T. Isemura, "Gendai Koshitsugaku no Tembo (Perspectives in Colloid Science, in Japanese)," Vol. 1, 177, Tokyo (1948).

## Experimental

The method used for measuring the surface potential is the vibrating plate method, which is the same as used in the previous work of our laboratory. The amplifier, which was constructed for present expriment, was a three stage amplifier of resistance coupling using valves 6C6, 6C6 and 42. The principle of measuring the potential is essentially the same as was originally devised by Zisman.(4) Surface tension was measured by a simple tensiometer of Du Nouy.

The substances studied are oxalic, maleic, fumaric, succinic, adipic, azelaic, sebacic and $\gamma\gamma'$-sulfonyl dibutyric acids. The first four acids are the purest commercial samples available. Those acids were repeatedly recrystallized from adequate solvents. Agreement of melting points with literature was satisfactory, excepting maleic acid. Recrystallized maleic acid used in our present experiment melted sharply at 138°. This melting point does not agree with the literature. However, Mr. I. Moritani found that a single crystal of pure sample of maleic acid melts sharply at that temperature.(5) Suberic and azelaic acids were prepared by usual method of oxidation of castor oil with nitric acid. The obtained azelaic acid was finally recrystallized from benzene(m. p. 106–108°). Suberic acid was fractionally recrystallized using ether repeatedly (m. p. 138–140°). Sebacic acid was prepared also form castor oil by the procedure presented by Sugino and his collaborators,(6) and was recrystallized from 15% alcohol (m. p. 131°). $\gamma\gamma'$-sulfonyl dibutyric acid was a pure sample which was given by Prof. S. Murahashi in this Institute.

All measurements were made at room temperature without special regulation of temperature. However, one series of experiments were made within a range of temperature as small as possible, and the variation of temperature never exceeded more than 2°.

## Results

The surface potentials observed are shown in Fig. 2 and Fig. 3, and the surface tensions of respective solutions are shown in Fig. 4. In these figures solid lines are the results for aqueous solutions and chain lines are the results for acidulated solution by 1/100 *N* hydrochloric acid. In Fig. 2, the curves for monocarboxylic fatty acids, *i. e.* butyric, valeric and *iso*-caproic acid which were already reported in the previous paper are also shown for comparison. Values of surface potential in relatively low concentration region of respective acids could not be determined as accurately as in rather concentrated region of the solutions. The parts of curves shown in dotted lines are the parts with somewhat less reliability than that of solid or chain lines. In these parts the potential fluctuated to some extent. Later the cause of the fluctuation of surface potential data in such concentration region will be thoroughly discussed.

Dicarboxylic acids which have relatively short carbon chain such as oxalic, maleic and fumaric acids gave always negative surface potential all over the concentration range examined up to saturation. With oxalic, maleic and fumaric acids, the potential reached to −140, −150 and −129 millivolts respectively at the concentration of nearly saturated solution. Succinic and

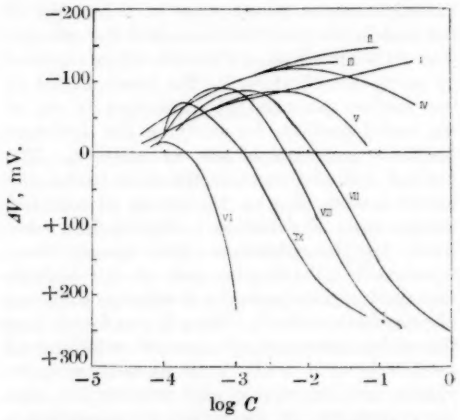


Fig. 2.— I, oxalic acid (11°C.); II, maleic acid (8°C.); III, fumaric acid (8°C.); IV, succinic acid (8°C.); V, adipic acid (10°C); VI, sebacic acid (10°C.); VII, n-butyric acid; VIII, valeric acid; IX, *iso*-caproic acid.

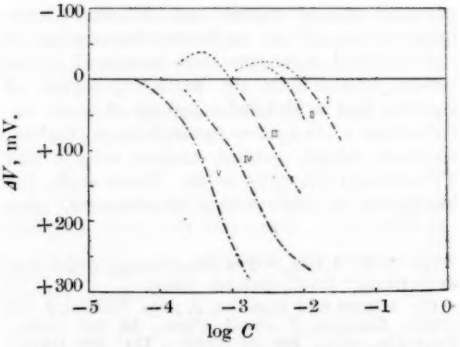


Fig. 3.— I, adipic acid (11°C.); II, $\gamma\gamma'$ sulfonyl dibutyric acid (14°C.); III, suberic acid (13°C.); IV, azelaic acid (14°C.); V, sebacic acid (17°C.).

(4) Zisman, *Rev. Sci. Instrument*, **3**, 7 (1932); Yamins and Zisman, *J. Chem. Phys.*, **1**, 656 (1933).

(5) Private communication.

(6) K. Sugino and collaborators, *J. Soc. Chem. Ind. Japan*, **44**, 706 (1941).

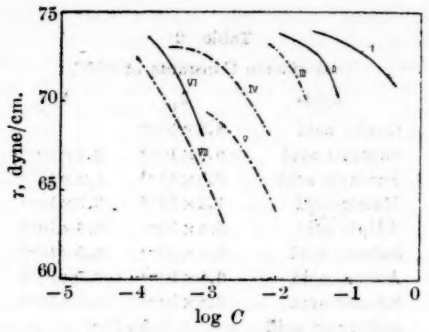


Fig. 4.— I, maleic acid (10°C.); II, adipic acid (9°C.); III, adipic acid+$N$/100 HCl (11°C.); IV, suberic acid+$N$/100 HCl (16°C.); V, azelaic acid+$N$/100 HCl (17°C.); VI, sebacic acid (10°C.); VII, sebacic acid+$N$/100 HCl (17°C.).

$$\Delta V = 4\pi n\mu,$$

with the aid of Gibbs' adsorption formula and our surface tension concentration curves. In the above equation $\Delta V$ is surface potential, $\mu$, the vertical contribution of dipole moment of the surface molecule and $n$, the number of adsorbed molecules per unit area. The vertical contributions of surface moments of suberic, azelaic and sebacic acids and their molecular area on the surface of solution at different surface potentials were tabulated in Table 1. The data of the area occupied and vertical component of dipole moment of adsorbed solute were shown as a function of surface potential and calculated assuming that both the activity coefficient and the dielectric constant were equal to unity.

Table 1

Molecular Area and Vertical Component of Dipole Moment with Respect to Surface Potential of Solutions of Dicarboxylic Acids
(calculated as activity coefficient = 1 and dielectric constant = 1)

| Surface potential $\Delta V$ (mV.) | | 280 | 240 | 200 | 160 | 120 | 80 | 40 |
|---|---|---|---|---|---|---|---|---|
| Molecular area (Å²) | Suberic acid | | | | 124 | 140 | 206 | 310 |
| | Azelaic acid | | 92 | 160 | 220 | | | |
| | Sebacic acid | 68 | 92 | 120 | 130 | 170 | 190 | 240 |
| Vertical component of dipole moment per each polar group (Debyes) | Suberic acid | | | | 0.27 | 0.23 | 0.22 | 0.17 |
| | Azelaic acid | | 0.30 | 0.37 | 0.47 | | | |
| | Sebacic acid | 0.25 | 0.30 | 0.33 | 0.28 | 0.27 | 0.20 | 0.12 |

adipic acids in aqueous solution also gave always negative potential, however, the potential tends to be less negative if the concentration of the solution approaches to saturation. Sebacic acid gave negative potential in very dilute solution but changed the sign of potential in more concentrated solution. With this acid, surface potential curve plotted as a function of logarithm of concentration (mol/l. )of the acid was similar to the curves of monocarboxylic fatty acids which were mentioned above. The potentials were generally shifted to more positive values when hydrochloric acid was added to the solution and maximum negative potentials were markedly suppressed. Maximum surface potentials of various dicarboxylic acids were not similar as shown in monocarboxylic acid and the more positive potential was observed the longer the carbon chain of the acid. Maximum potential observed in our experiments for adsorbed layer of solutions was 280 mV. for 0.016 mol solution of sebacic acid at 17° acidulated with hydrochloric acid so as to become 0.01 $N$ with respect to hydrochloric acid. The vertical contribution of surface moment of the adsorbed acid was calculated by Helmholtz equation,

## Discussion

From the results just mentioned above, it will be noticed that the surface potentials of solutions of dicarboxylic acids near the concentrations of their saturated solutions became more positive value with increase of the length of hydrocarbon chain in the acid molecules. This is a remarkable difference between the results obtained with dicarboxylic acids and those with monocarboxylic acids. With monocarboxylic acids such as butyric, valeric and *iso*-caproic acids, solutions of these acids showed almost the same positive potential, *i.e.* *ca*, 240 mV. in the concentration ranges near saturation. If the solutions of dicarboxylic acids of moderate carbon chain length were acidulated with hydrochloric acid, the surface potential was generally shifted to more positive value. Then, the curves of surface potential versus logarithm of concentration were almost parallel as shown in Fig. 3, and from the figure it is obvious that about one third concentrated solution is sufficient to attain a certain value of surface potential if the acid has one more $CH_2$ group in the chain. This fact is analogous

to that found with monocarboxylic acids in the previous investigation. The work of adsorption is increased in the cases of dicarboxylic acids also, by a constant amount for each $CH_2$ group added to the chain, so that the relation of every $CH_2$ to the water surface is the same, whatever its position in the chain.

In the previous paper, we inferred that the reversal of sign of surface potential that was observed at high dilution of solution may be the characteristic phenomena for the adsorbed layer of gaseous film type, caused either by molecular orientation, that is lying flat on the surface, or by the dissociation of adsorbed acids. Since the acids investigated in the present work are dicarboxylic and have two polar groups at both ends of the molecule, two water soluble polar groups would naturally make the molecule lie flat, if the surface potential is defined principally by the orientation of the adsorbed molecules, the potentials of solutions of these acids will not reverse the sign at all, and change steadily to a saturation value. With oxalic, maleic and fumaric acids, which have relatively large dissociation constants, this is the case. With dicarboxylic acids of longer chain than adipic acid, however, the reversal of the sign of the potential was observed just as with monocarboxylic fatty acids. Thereupon, these facts cannot be attributed simply to the change of orientation of the adsorbed molecules. Further, if the reversal of potential was caused by the orientation, the calculated vertical contribution of the dipole moment of the molecules of dicarboxylic acids, which lie on the surface of solution, should be independent of the concentration of the solution and of the length of carbon chain of the acids. These inferences were not supported with our experimental results. On the other hand, if we assume the reversal of surface potential are caused by the dissociation of the acid, the experimental results will be explained without any difficulty. In acidulated solutions, the dissociation of dicarboxylic acids will be suppressed and the potential of the surface was shifted to more positive values in comparison with that of non-acidulated solutions. In very dilute range of concentration of the solution, number of molecules of adsorbed acid might be relatively few and a large proportion of the adsorbed acid should be ionized. Hence, surface potential of the solution may be reversed the sign.

These inferences would readily be comprehended by comparing the surface potential curves in Fig. 2 and Fig. 3 with dissocation constants of respective acids tabulated in Table 2. Except oxalic, maleic and fumaric acids which have relatively high dissociation constants, all acids investigated have the first dissociation constants of the order of magnitude of $10^{-5}$. With the aid of Ostwald's dilution law, the degree of dissociation of dilute solutions of various concentrations can readily be calculated and rough estimate at two different dissociation constants would have values as shown in Table 3. Since hydrochloric acid is practically dissociated completely in 1/100 *N* solution, the dissociation degree of the acids, having dissociation constant of the order of magnitude of $10^{-5}$, will be as much as $3\times10^{-3}$.

Surface potential changed its value to some extent with time after making surface on the solution according to the kind and concentration of acid. The values plotted in Figs. 2 and 3 were those within one minute after making new surface. The potentials in the range of negative value tend to more negative value with time. It has already been pointed out by some authors that surface tensions of solution of certain substances changed with time,[7] and at least five minutes were required to attain steady values of surface tension with all aliphatic acids above *n*-butyric acid. Time required to obtain equilibrium surface tensions

Table 2

Dissociation Constants at 25°C.

| Acids | $k_1$ | $k_2$ |
|---|---|---|
| Oxalic acid | $3.8\times10^{-2}$ | |
| Succinic acid | $6.5\times10^{-5}$ | $2.3\times10^{-6}$ |
| Fumaric acid | $9.3\times10^{-4}$ | $1.8\times10^{-6}$ |
| Maleic acid | $1.2\times10^{-2}$ | $3.9\times10^{-7}$ |
| Adipic acid | $3.8\times10^{-5}$ | $2.4\times10^{-6}$ |
| Suberic acid | $3.0\times10^{-5}$ | $2.5\times10^{-6}$ |
| Azelaic acid | $2.6\times10^{-5}$ | $2.7\times10^{-6}$ |
| Sebacic acid | $2.8\times10^{-5}$ | $2.6\times10^{-6}$ |
| *n*-Butyric acid | $1.5\times10^{-5}$ | |
| *n*-Valeric acid | $1.5\times10^{-5}$ | |
| *iso*-Caproic acid | $1.5\times10^{-5}$ | |

(cited from the Beilstein's Handbuch.)

Table 3

Degree of Dissociation Calculated from Ostwald's Dilution Law

| Dissociation constant | Concentration, *N* | | | | |
|---|---|---|---|---|---|
| | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ |
| $1.5\times10^{-5}$ | 0.012 | 0.038 | 0.12 | 0.31 | 0.68 |
| $2.5\times10^{-5}$ | 0.016 | 0.046 | 0.14 | 0.39 | 0.77 |

(7) H. H. King, *Kansas State Agr. Col., Agr. Exp. Station Techn. Bull.*, No. 9, (1922); Addison, *Nature*, **156**, 600 (1945).

increases very rapidly with chain length of acids. Of course, the cause for the change of surface potential with time might be the same with that for surface tension and attributed probably to slow adsorption of solute. Ions of acids are exceedingly more soluble than undissociated molecules. The latter will immediately be adsorbed. While, ions which have hydrocarbon of moderate length and are considerably soluble in the solution, would be adsorbed very slowly. The rate of adsorption of ions must be slower than that of undissociated molecules and depends on the length of hydrocarbon chain. If such ions of moderate hydrocarbon chain reached to surface, they would be captured by surface layer and partly cambined with hydrogen ion and converted into undissociated molecule according to relatively high concentration of such weak acid in the surface phase. Nevertheless, actual concentration of negative ions is gradually increased and surface potential effectively changes to more negative. With acids of sufficiently long chain, solubility of those acids would be decreased markedly and the acids might be often practically insoluble, and the adsorbed layer, which covers the surface of solution completely or surface monolayer closely packed, would be made exclusively of undissociated molecules. Hence the surface potential is positive and has no bearing on time. The surface films of insoluble fatty acids such as stearic and myristic acids, of which dissociation constants are the order of magnitude of $10^{-13}$, give generally positive surface potential. Even with such acid of low dissociation constant as myristic acid, however, the change of sign of surface potential was observed recently by Guastella and Michel[8] at extremely dilute surface concentrations. They found with myristic acid that surface potential changes to negative for molecular area from 1000 to 30000 Å.$^2$ They concluded that the results obtained by them at high dilution appeard to indicate that the molecules of myristic acid turn their negative pole upwards. On the other hand, with the concentrated solution of acids which have moderate length of carbon chain and show some solubility, surface layer is completely covered with preferentially occupied undissociated molecules and leaves no space for belated ions. Under these circumstances also, the potential is always positive and does not change with time.

Each molecule of sebacic acid, dicarboxylic acid of ten carbon atoms, occupied an area of 68 Å.$^2$ on the surface of its $10^{-3}$ $N$ solution, whose surface potential of which was 280mV. Calculated from these figures, the vertical component of dipole moment assigned to each polar group was 0.25 D.U. This is nearly identical with the value of vertical contribution of dipole moment of monocarboxylic acid at higher concentration or of monolayer of insoluble saturated fatty acid. In this case the molecule may be anchored by two carboxylic groups at both ends, neverthless, the hydrophobic hydrocarbon chain is repellent to aqueous phase. So that, the molecule probably bends somewhat at the middle of the molecule as shown in Fig. 1. This conclusion is not strange at all. J. T. Davies measured force area and surface moment area curves for the monolayers of some long chain diesters containing the same number of carbon atoms.[9] A plot of areas at the same surface pressure against the number of carbon atoms in each end chain was drawn. He found a maximum close to the point where the number of carbon atoms in the end chains is equal to the number in the middle of the molecule. These results together with our own concerning with sebacic acid are both equally understood, if the molecule is considered to bend at its middle. It must be emphasized, however, that the reversal of the sign of surface potential of dicarboxylic acid is not always connected with the bending of molecule. This will be clearly understood by the fact that $\gamma\gamma'$-sulfonyl dibutyric acid, which has one more polar group of hydrophilic nature in the middle of molecule and for that reason cannot bend the chain molecule, reversed the sign of the surface potential at high dilution. For these reasons, it is believed that the reversal of sign of surface potential would be caused mainly by the dissociation of some weak acid. Some aliphatic non-ionizable substances are to be investigated.

## Summary

Surface potentials of solutions of some dicarboxylic acids were measured by vibrating plate method. Dicarboxylic acids of short chain such as oxalic, maleic, fumaric and succinic acids gave always negative potential. However, dicarboxylic acids of moderate chain length gave positive potential at high concentration. While, with these acids, the potential changed to more negative at high dilution as in the cases of monocarboxylic acids. The values of potential were shifted to more positive values when the

(8) J. Guastella and Michel, *Research (London), Suppl., Surface Chemistry*, 127 (1949).

(9) J. T. Davies, *Trans. Faraday Soc.*, **44**, 909 (1948).

solutions were acidulated by hydrochloric acid. Such reversal of sign of surface potential might be attributed mainly to the dissociation of the acid and not to the molecular orientation. Molecule of sebacic acid adsorbed on the surface of nearly saturated solution bends probably its hydrocarbon chain at the middle of the molecule and it behaves as two adsorbed molecules of monocarboxylic acid in regard to molecular area and surface moment.

The authors' thanks are due to Prof. S. Murahashi who kindly gave them the pure sample. The cost of this research has been partly defrayed from the Scientific Research Encouragement Grant from the Ministry of Education, to which the authors' thanks are due.

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# Fluorometric Microdetermination of Uranium. II. Extraction of Uranium

By Masaki NAKANISHI[1]

(Received March 9, 1950)

Various properties of the fluorescent material of sodium fluoride containing a small quantity of uranium were investigated in the previous paper with the same title. To prepare the fluorescent material for the determination, it is required to extract uranium from the sample for analysis in an adequate form of compound.

Precautions must be taken lest any loss of uranium should be made throughout the procedures, and since the fluorescent materials are generally liable to be affected by impurities, they should always be guarded against such effects. But as was mentioned in the previous paper, the fluorescence of sodium fluoride activated by uranium is fortunately less affected by impurities, so far as the analytical application is concerned. For example, heavy metals and insoluble matters are made ineffective in the determination, if their amounts are decreased at least to the amount comparable with that of the uranium present with them. According to the writer's experiences, this is satisfactorily accomplished by the careful operations in the chemical separation procedures.

It is attempted in the present paper to survey each individual process for extracting a minute quantity of uranium from any sample, and to establish a method as satisfactory as possible.

## 1. Outline of the Extraction from the Sample

In dealing with the solid sample, it must be first brought into a solution of uranyl salt. For example, a rock sample is ground and fused in a platinum crucible with sodium carbonate, then extracted with hot water.

This treatment must be repeated at least three times, for only one process does not suffice to perfect the extraction. The extraction with hot water instead of any acid makes the following scheme simpler, because many other metals are separated from uranium and the latter is in general completely brought into the solution.

The following represents the outline of extraction method of uranium in usual natural substances, in which are included solutions derived from rock samples, sea-water, hot-spring water, etc. In case when the sample contains unfamiliar constituents, a special scheme of the analysis must be chosen, but this consideration is omitted here for simplicity.

The application of the fluorometric method of analysis is practically limited to the solution, in which the uranium content is so small that the element is not quantitatively precipitated by any direct way. To precipitate uranium from such a dilute solution, iron hydroxide is effectively employed as carrier or co-precipitator, as is often the case with the precipitation of a small amount of substance.

The extract from sodium carbonate fusion is carefully treated with dilute hydrochloric acid and boiled to expel a large amount of carbon dioxide. The resulting solution free from carbonates is evaporated on a water-bath to dryness, and silica is, if necessary, removed as usual from this residue. Since organic matters and carbonates seriously interfere with

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the coprecipitation of uranium with iron, it is better in the case of an aqueous sample, *e. g.*, natural water, to begin with the evaporation to remove silica and then to destroy the organic substances with nitric acid or a mixture of nitric acid and sulfuric acid.

The residue is dissolved into a clear solution. Add ammonium chloride and an adequate amount of ferric chloride solution (corresponding to about 20 mg. of $Fe_2O_3$), boil for a few minutes, then add ammonia containing no trace of carbonate. Almost all uranium is coprecipitated with iron hydroxide. The precipitate is heated for a while with its mother liquor, which is followed by filtration and washing with a hot ammoniacal ammonium chloride solution containing no trace of carbonate. To complete the precipitation of uranium, this procedure is repeated three times.

Some considerations will be made in the following in connection with these treatments and the coprecipitation phenomenon.

In the next stage of analysis, uranium coprecipitated must be separated from iron and other possible impurities, but this will be detailed in the following papers.

## 2. Required Amount of Iron for Coprecipitation

The following experiment provides some knowledges how much ferric salt will be needed to precipitate the minute quantity of uranium together with iron hydroxide.

Different quantities of ferric chloride solution were added to 50 ml. of uranyl nitrate solutions, each containing $1.00 \times 10^{-5}$ g. of uranium. In these mixtures, precipitation with ammonia was performed, and the uranium content of each precipitate was determined by the fluorometric method described later.

Table 1

| $FeCl_3$ added, mg. as $Fe_2O_3$ | Amount of uranium in each bead, $10^{-7}$ g. | | Observed / Expected |
|---|---|---|---|
| | Expected | Observed | % |
| 1 | 8.78 | 8.27 | 94 |
| | | 9.18 | 104 |
| 3 | 9.78 | 9.85 | 101 |
| | | 8.75 | 89 |
| 10 | 8.66 | 8.85 | 102 |
| | | 8.27 | 95 |
| | | 9.18 | 106 |

Table 1 represents the experimental results, which prove that 1 mg. as well as 10 mg. of iron is equally sufficient to precipitate about $10^{-5}$ g. of uranium. The experiment with the smaller amount of iron than 1 mg. was omitted, for it is considered unavailable in practical cases.

## 3. Amount of Uranium not Precipitated

It is generally recommended to repeat the procedure two or three times in precipitating a small amount of substance with carrier. The next experiment reveals the efficiency, with which uranium precipitates with a relatively large amount of iron.

To the hot 100 ml. solution containing $2 \times 10^{-6}$ g. U and 20 mg. $Fe_2O_3$ in the forms of uranyl nitrate and ferric chloride respectively, ammonia was added, and the precipitated iron hydroxide was filtered. Then the amount of uranium not precipitated was determined in the filtrate by means of the fluorometric method.

As a result of this experiment it was observed that only a trace of uranium was found in the filtrate and almost all of it was dragged down with the hydroxide precipitate. Approximately from 0.3 to 0.4% of the uranium in the original solution gets rid of the coprecipitation and remains in the filtrate. The values determined were not precise in this experiment, for the uranium content of each bead is in the order of $10^{-9}$ g., corresponding approximately to the lower limit to produce faint fluorescence.

Since the error in the determination of this fluorometric method amounts to about 10%, a single coprecipitation procedure of uranium with iron will serve in practical cases. But the coprecipitation procedure is preferably repeated three times as a rule, for it is suspected that the efficiency will be lowered if the solution contains various other substances.

## 4. On Adsorption

The iron hydroxide precipitate adsorbs other impurities, as is well known, on account of its colloidal property. In the experiment mentioned, therefore, special precautions will be needed in the operations, if uranium is precipitated by mere adsorption with iron hydroxide. But it is valid from the following experiments that the adsorption, if any, is neglected, and the suitable condition will be shown thereby.

**a. At the room temperature.**—The hydrochloric acid solution of uranyl nitrate containing $1.05 \times 10^{-5}$ g. U was boiled to expel carbon dioxide, left standing to coolness, and ammonia was added, where no noticeable precipitate was produced. On the other hand, the hydrochloric acid solution of ferric chloride containing 2 mg. $Fe_2O_3$ was kept boiling for a

while, and after cooled to the room temperature iron hydroxide was precipitated with ammonia. The two solutions were mixed together immediately after adding ammonia (volume of the resultant solution being about 100 ml. and the concentration of ammonia 1 *N*), shaked for 5 minutes and filtered through a hard filter paper under suction. The precipitate was dissolved in hot dilute hydrochloric acid and after iron being separated uranium was determined by the fluorometric method. The numerical data are given in Table 2 (a). The amount of uranium apparently included in the precipitate reaches as much as 8.10% of that taken. This fact gives an impression that a part of uranium in the solution is adsorbed on the surface of iron hydroxide precipitate. But in the above treatment, iron precipitate had been completed before mixing with uranium solution, and the filtration followed soon after mixing. Therefore the so-called occlusion or the compound formation may not be the case. Considering that uranium belongs to those elements which are precipitated in an ammoniacal medium, it is suspected that from such a dilute solution uranium may form an unnoticeable precipitate remaining on the filter paper. To make this clear, a similar experiment was made with the solution which contains uranium only, and is not added with iron precipitate. After the filtration, the filter paper was ignited to ash, and the uranium content in the ash was estimated as before. 12.2% of the total amount of uranium taken was found as given in Table 2 (b).

Table 2

| | Uranium taken, $10^{-7}$ g. | $FeCl_3$ added, mg. as $Fe_2O_3$ | Temperature, °C. | Uranium found in the precipitate, $10^{-7}$ g., and % to the total | |
|---|---|---|---|---|---|
| (a) | 105.4 | 2 | 20 | 8.53 | 8.10% |
| (b) | 〃 | none | 〃 | 12.9 | 12.2 〃 |
| (c) | 10.54 | 20 | at boiling | 1.39 | 13.2 〃 |
| (d) | 〃 | 〃 | 〃 | 1.33 | 12.6 〃 |
| (e) | 〃 | 〃 | 〃 | 1.90 | 18.0 〃 |
| (f) | 〃 | none | 〃 | 1.69 | 16.0 〃 |

It seems contradictory that in the experimental results the amount of uranium remaining with the iron precipitate ((a) in Table 2) is smaller than that remaining on the filter paper only ((b) in Table 2) where no iron precipitate was present. But the state of uranium precipitate from such a dilute solution might delicately depend upon the conditions in the procedure, and it has therefore almost no meaning to discuss the difference.

In conclusion, the adsorption of uranium to iron precipitate is practically negligible at the room temperature, and its contribution, if any, is not so large as to explain the fact that nearly all of uranium in the solution is precipitated with iron hydroxide as described in the above section (3).

**b. At the higher temperature.**—Since in practical cases the procedure to precipitate a small amount of uranium with iron is performed at the hot condition, the examination on the adsorption at a higher temperature is more significant than that at a lower temperature. In fact, it is known that the iron hydroxide precipitate changes its structure by aging at a higher temperature,[2] and from this fact further examinations are required to see whether uranium is adsorbed to iron hydroxide precipitate at the hot condition or not.

**Experiment 1.**—A 50 ml. solution containing $1.05 \times 10^{-6}$ g. U as uranyl nitrate and 1 g. of ammonium chloride was heated to boiling and ammonia was added, where no visible precipitate took place. On the other hand another 50 ml. solution containing 20 mg. $Fe_2O_3$ in chloride form and 1.5 g. of ammonium chloride was heated to boiling and ammonia was added. The latter solution with iron precipitate in it was immediately transferred to the ammoniacal solution of uranium, the mixture being heated again with stirring for 1 minute, and then filtered under suction without washing. The precipitate remaining on the filter paper was dissolved in hot dilute hydrochloric acid and uranium was determined ((c) in Table 2).

**Experiment 2.**—After mixing two ammoniacal solutions of uranium and iron treated as before, the combined solution was kept on heating with the precipitate in it for 10 minutes. Other treatments were the same as the above. ((d) in Table 2).

**Experiment 3.**—Before mixing with an ammoniacal solution of uranium, iron hydroxide precipitate was aged with the mother liquor for 10 minutes at the boiling temperature. Other details were the same as in Experiment 1. ((e) in Table 2).

**Experiment 4.**—The same treatment as in Experiment 1 was carried out without iron precipitate, to determine the amount of uranium remaining on the filter paper. ((f) in Table 2).

As given in the table, the amounts of uranium

(2) A. Krause, *Z. anorg. allgem. Chem.*, **176**, 398 (1928); **204** (1932); **206**, 328 (1932); **211** (1933); **219**, 213 (1934).

which remain with the precipitate are not constant, but they are in the same order of magnitude as that obtained when uranium solution is treated without iron. Besides, these values are dependent to a large extent upon the conditions of the precipitation procedure. When uranium is precipitated alone, the precipitate of small sizes will perhaps remain adsorbedly on the filter paper.

Conclusions from the experimental results above mentioned are as follows. The precipitation phenomenon of uranium with iron is not attributed to the mere adsorption. Even the adsorption should take place, it may be limited only to a smaller part, and may not play an important role. It is reasonably concluded that a larger part of uranium will be coprecipitated with iron in some structural binding. In consequence, this procedure of precipitation is effectively made at a convenient hot condition, and the washing will not cause any loss of the element.

### 5. On Aging

If uranium should combine loosely with iron hydroxide precipitate, it may be possible that the former is let free from the latter and again gets into the solution during digestion at the high temperature. Now that uranium itself is slightly soluble in an ammoniacal medium, there will be almost nothing to apprehend, but an exact proof for it is required, because the amount of uranium taken is negligibly small for the ordinary chemical manipulations. The following experiments will meet the purpose.

A 100 ml. solution containing $1 \times 10^{-6}$ g. U in nitrate form and 20 mg. $Fe_2O_3$ in chloride together with 2.5 g. of ammonium chloride was acidified with hydrochloric acid, boiled for 1 minute, and 10 ml. of 6 *N* ammonia was added. In one experiment the precipitate of iron hydroxide obtained was immediately filtered, while in the others the precipitate was digested at 95° for 3, 10 and 60 minutes respectively before filtration. After filtered in each case uranium remaining in the filtrate was determined by the fluorometric method. The values obtained were very small, which correspond to about 0.5% of the total amount taken in each case. According to our present method of procedures, an amount of uranium close to $10^{-9}$ g. gives too weak a luminescence to obtain the exact value.

This amount of uranium which gets rid of the coprecipitation with iron, however, agrees satisfactorily with that which remains in the solution after a single coprecipitation procedure as described before (section 3). Therefore it is concluded here that there is no loss even if we keep the precipitate heated with its mother liquor as long as an hour. Furthermore, this procedure of boiling gives a favorable result, for aging for a while at the boiling temperature makes the iron hydroxide precipitate more filterable.

### Summary

A minute amount of uranium suitable for the fluorometric method of determination is effectively precipitated from the solution together with hydrous ferric oxide as co-precipitator. The efficiency of co-precipitation seems to be nearly perfect for the purpose, and the adsorption and the digestion effect were observed in particular from the analytical standpoint.

The writer is greatly indebted to Professor Kenjiro Kimura for his kind guidance in the course of this work.

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# Fluorometric Microdetermination of Uranium. III. Isolation of Uranium and Preparation of the Fluorescent Material

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(Received March 9, 1950)

This series of reports aims to tell in detail the fluorometric method of determination of uranium, and the procedure fitted for the estimation of such a small amount of the element as we often encounter in the analysis of some geochemical samples. The most suitable amount to deal with is within the limit from

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$10^{-5}$ g. to $10^{-7}$ g. of uranium.

The first step in treating the sample is just the same as in the ordinary chemical analysis, the details of which have been mentioned in the preceding paper. To extract uranium from the solution, ferric hydroxide precipitate is effectively used as carrier. A minute quantity of uranium is completely coprecipitated with this carrier, and this phenomenon has been reported in detail from the analytical point. In this report the separation of uranium from iron and other impurities will be taken into account.

## 1. Separation from Iron

The minute quantity of uranium in the solution from the sample is concentrated in the iron precipitate as was described in the foregoing paper. The coprecipitation procedure must be repeated at least three times for completeness.

If the original solution contains those elements which produce precipitates in the ammoniacal medium, they will naturally contaminate the precipitate of iron. Besides, the colloidal property of the precipitate will also cause adsorption or inclusion of some elements or compounds. As it is, of course, profitable for the following processes to avoid the latter sort of contaminations, the iron precipitate containing uranium is dissolved in hot dilute hydrochloric acid, and reprecipitated along with uranium. In case of the relatively concentrated solution of alkali or alkaline earth metals, e. g., solutions obtained from rocks, minerals, sea water, etc., the re-precipitation is necessary.

In the treatment of ordinary natural substances, except some special ones, impurities by coprecipitation are eliminated in the next stage of procedures, and a smaller part of them remaining with uranium affects, according to our experiences, not so much as to interfere in the fluorometric determination.

The total precipitate of iron hydroxide containing a small amount of uranium obtained from the original solution is dissolved out, as soon as possible after filtration and washing in hot dilute hydrochloric acid. No trace of uranium remains adsorbed to the filter paper. And from this solution the iron hydroxide is again precipitated together with uranium. A little ferric chloride solution corresponding to a few milligrams of $Fe_2O_3$ is added to the filtrate, and the recovery of uranium is repeated in the same way as in the preceding paper. These precipitates of iron containing uranium too are dissolved in hydrochloric acid.

From this acid solution of iron and uranium, the most part of the former is separated from the latter, with the aid of ammonia in the presence of ammonium carbonate.[2]

In this procedure of separating iron, ammonium chloride is added to the acid solution which is almost neutralized with ammonia. Keeping the solution at about 60°, ammonium carbonate and ammonia are added, and the solution is kept warm with the iron precipitate in it for several minutes. The decomposition of ammonium carbonate begins here if the temperature rises higher than about 80°. The iron precipitate is now filtered off, which contains almost no uranium if the directions are followed.

But it is not easy to wash the precipitate obtained here, for it has been formed at a rather lower temperature. On this account the washing is omitted in this stage, and the precipitate is re-dissolved in hot dilute hydrochloric acid, and the procedure is repeated as given to complete the dissolution of uranium.

Since the filtrate occasionally contains a trace of iron, it is treated with hydrogen sulphide, and the precipitate formed is filtered off.

## 2. Separation of Other Impurities

The filtrate separated from iron precipitate contains, besides all of uranium taken, some impurities which behave in the same way as uranium in the coprecipitation with and separation from iron. But these give almost no unfavorable effects on the determination so far as common samples are dealt with. In cases where the samples are rich in beryllium, rare earth metals, zirconium, thorium, etc., special procedures of chemical separation are necessary in addition to the simpler one described in the foregoing sections.

The filtrate is cautiously made acid with hydrochloric acid, and evaporated in a porcelain dish. If any heavy metals are still present, these may be precipitated with the help of organic reagents such as 8-hydroxyquinoline in the presence of ammonium carbonate. Here the repeated operations are necessary to prevent the precipitation of uranium compound.

The filtrate is gently evaporated in a porcelain vessel. The residue mostly consists of ammonium salts, and these must be eliminated. If these are heated directly, a little loss of uranium is likely to occur. So the ammonium

(2) The ammonium carbonate reagent is prepared as follows, 40 g. of the commercial reagent is dissolved in 1 l. of 1 *N* ammonia, warmed at about 60° for 10 minutes, and filtered if necessary.

salts are preferably removed as follows: Add concentrated nitric acid to the residue, and the vessel covered with a watch glass is warmed on a water-bath. After the violent reaction has ceased it is further evaporated to complete dryness.

That the latter method is superior to the former is evident from the next experiment. The solution containing $1.00 \times 10^{-6}$ g. U as nitrate and 1 g. of ammonium chloride was evaporated to dryness. The residue obtained was heated into fume, and the fluorometric method was applied to determine the remaining uranium. In comparison with this, the ammonium chloride residue was decomposed with nitric acid, and the remaining uranium determined. Table 1 represents the results obtained.

Table 1

| | Amount of uranium, $10^{-6}$ g. | | |
|---|---|---|---|
| | Calculated | Observed | |
| By direct heating | 1.00 | 0.72<br>0.91<br>0.73<br>0.84<br>0.97<br>0.79 | mean: 0.83 |
| Decomposition with nitric acid | 1.00 | 0.73<br>0.89<br>1.03<br>0.94<br>1.02<br>0.97<br>1.10<br>0.90 | mean: 0.96 |

It is obvious from the table that the operation to remove the ammonium salts by fuming gives rise to a loss of uranium, and the loss may be due either to the actual loss that a minute quantity of uranium is spattered with the fume of ammonium salts, or to the apparent loss that uranium is transformed by heating into an insoluble compound and is not mixed up into the fluorescent material.

The residue after decomposing the ammonium salts in which the total amount of uranium is contained is very small, if the directions are followed with care, and such a small amount of contamination, does not interfere in the fluorescence test.

Nitric acid is used again, if necessary, to decompose the chlorides completely. Then the content of the vessel is transferred into a small platinum dish, moistened with nitric acid and evaporated again on a water-bath. Mixed with sodium fluoride, the final residue is next brought into the fluorescent material as given in the section (3).

The impurities in the final residue, if they are much, may interfere with the formation of the normal fluorescent material as a result of contamination. Therefore it is required to pay attention to every stage of the operation, and to perform the separation of impurities if necessary. As for vessels, porcelain of good quality is preferable to glass, especially for the long-during evaporation, since the dissolution of constituents of glass is thus avoided. The platinum vessel is used after chlorides are decomposed with nitric acid.

### 3. Preparation of the Fluoresent Material

**a. Mixing with sodium fluoride.**—The total amount of uranium is now brought together in a platinum dish. About 150 mg. of solid sodium fluoride and a little water are added into it. Then the solution is evaporated to complete dryness on a water-bath. The dried mass of sodium fluoride in which uranium is contained is scraped off and preserved in a dessicator. Pour several ml. of water into the dish to dissolve the possible remainder of uranium salt. Then add more 50 mg. of sodium fluoride and evaporate to dryness.

The sodium fluoride masses obtained by the successive operations are gathered together, and heated gently in a platinum dish to remove the moisture completely. The obtained powder mixture is preserved in the desiccator and the fluorescent beads are made from this mixture as will be mentioned in the following section.

Speaking from the writer's experiences, it is not profitable in the last operation of mixing to use hydrofluoric acid instead of water for the following reasons. (i) First, if hydrofluoric acid is used, a molecular compound $NaF \cdot HF$ is formed, from which hydrogen fluoride is liberated on heating and the mechanical loss of the fluorescent material takes place at times in the preparation of fluorescent beads. Besides, the beads prepared from such material become much smaller than expected, and do not develop into a disk-shaped bead. (ii) Secondly, there is a fear that impurities of the acid are mingled, while it is rather an undesirable work to purify the acid. (iii) Moreover, the writer experienced no objection in using water instead of hydrofluoric acid.

**b. Preparation of fluorescent beads.**—Weigh the total of the dried powder mixture of uranium and sodium fluoride, and make it homogeneous in a mortar. The fluorescent bead is prepared from a portion of this mixture

on a platinum loop. But as it is difficult to treat such a powdered sample, this is conveniently pressed into a small tablet of about 20 mg. in weight. Each tablet is weighed exactly.

The preparation of the fluorescent bead runs as follows: A tablet of a known weight is put on the clean platinum loop, and gradually heated by the burner flame to clear fusion in the end. This state of fusion is kept for 5 minutes in the oxidizing flame. Both sides of the disk-shaped bead are to be equally heated, and this condition must be the same for every bead. After 5 minutes' fusion, the bead is quickly cooled down rotating the shaft attached to the loop, by which procedure the surface of the bead becomes flat and suitable for the determining the fluorescence intensity.

To obtain a disk-shaped bead, the proportion of the loop diameter to the amount of the powder mixture is important, and this is decided by trials. The writer uses regularly the loop of 2.5 mm. diameter for about 20 mg. of the mixture.

The time of ignition in the burner flame is fixed to 5 minutes. The intensity of fluorescence depends much upon the time of heating and, during the first few minutes, the longer the time is, the more intense the fluorescence becomes, since it takes some time for heating to form a proper fluorescent material. But the intensity increases very gradually with time when heated for more than several minutes, which means that there is a relative increase of uranium content in the bead due to the evaporation of sodium fluoride under heating. This is the reason why the duration of the heating treatment must be constant for every bead, and the heating for 5 minutes was chosen by trials.

The bead of the fluorescent material prepared is somewhat hygroscopic. It is not desirable to repeat the ignition since it might cause the change of the intensity. Therefore it is better to prepare the bead immediately before testing the intensity.

**c. Preparation of the standard beads.**— The intensity of fluorescence of the bead from an unknown sample is compared, as will be described in the following paper, with those of the standard beads, the uranium contents of which are already known. These stndard beads are prepared as follows: to the known amount of purified sodium fluoride in a platinum dish, add an adequate amount of the standard solution of uranyl nitrate, evaporate the mixture up to complete dryness and weigh the total. Make the mixture homogeneous in the mortar. From this prepare tablets of about 20 mg. each and reserve them. The uranium content of each tablet is known from its weight comparing with that of the total mixture. The following procedures of preparation of the standard beads go as in the case of the unknown samples.

## Summary

The procedures to isolate uranium, which is coprecipitated with the iron hydroxide, are related. The minute quantity of uranium obtained is mixed with sodium fluoride and the fluorescent beads for the fluorometric determination are made from the mixture.

The writer is indebted to Professor Kenjiro Kimura for his generous advice and criticism in the course of this investigation.

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# Influence of Substituent Groups on Nuclear Reactivity in Formations of Substituted Biphenyls through Reactions of Aromatic Diazo and Cognate Compounds with Aromatic Liquids. I. The Nitro Group[1]

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(Received June 19, 1950.)

Diazo hydroxides and *n*-nitrosoacetylarylamines decompose in an aromatic liquid to yield biphenyls according to the following formulae:

$$ArN_2OH + Ar'H \longrightarrow ArAr' + N_2 + H_2O,$$

$$ArN(NO)Ac + Ar'H \longrightarrow ArAr' + N_2 + AcOH.$$

The preparative aspects of these reactions have recently been fully reviewed by Bachmann and Hoffman.[2] As regards the mechanism of the formation of biaryls there has been amassed a considerable number of evidences to warrant the conclusion that the reaction takes place through a mechanism involving free aryl radicals which, having been produced by decomposition of diazo hydroxides or nitrosoacetylarylamines, react with aromatic compounds giving corresponding biphenyls. In support of the free radical mechanism is, among others, the observation that para and ortho substituted biphenyls are invariably formed in these reactions irrespective of the nature of the substituent groups, a fact which is unaccountable on the basis of the usual polar influences of substituents accepted as operative in the substitution by kationoid or anionoid reagents. Furthermore, it is striking that nitro group was found to exert its influence by activating the benzene nucleus to which it is attached in the substitution reaction by phenyl radical as was shown by the fact that nitrobiphenyls were obtained in a yield greater than methylbiphenyls in a competitive reaction in a mixture of nitrobenzene and toluene.[3]

With a view to further elucidating the nature of influences of substituent groups in the substitution by the aryl radical a series of analytical investigations of these reactions has been undertaken in this laboratory, and the results obtained from the investigations on the influence of the nitro group are described in the present report. By determining the ratio in which biphenyl and three isomeric nitrobiphenyls were formed by the reactions of diazobenzene hydroxide and nitrosoacetanilide respectively in a mixture of benzene and nitrobenzene the reactivities of the various nuclear positions of nitrobenzene relative to that of benzene could approximately be estimated.

## Results and Discussion

Careful fractionation of a reaction mixture from nitrosoacetanilide and nitrobenzene at 20° yielded a mixture of isomeric nitrobiphenyls in yields varying from 30 to 37% based on the nitrosoacetanilide utilized. From this mixture readily crystallizable *p*-nitrobiphenyl was separated as much as possible and weighed after recrystallization. As the isolation of the other isomerides turned out difficult, recourse was made to the estimation of *m*-nitrobiphenyl by titration with titanous chloride and subsequently with a bromide-bromate mixture according to the method, developed by Francis and Hill,[4] with a slight modification. Thus, it was found that *m*-nitrobiphenyl constituted as much as 23% of the original mixture, and the *p*-nitrobiphenyl which was isolated as a pure specimen amounted to 17%. The remaining 60% were regarded as composed of the whole of the ortho isomeride formed and of that part of para isomeride which could not be isolated.

When nitrosoacetanilide was decomposed in a mixture of nitrobenzene and benzene (in a molar ratio of 1:30:60), biphenyl and nitrobiphenyls were obtained in yields of 4.6 and 19.7%, respectively, on the basis of the nitrosoacetanilide used. It was found by the same

(1) This paper contains materials presented at the 2nd and the 3rd annual meetings of the Chemical Society of Japan held in Tokyo, April 1949, and in Kyoto, April 1950, respectively.

(2) W. E. Bachmann and R. A. Hoffman, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, 1944, p. 224.

(3) W. S. M. Grieve and D. H. Hey, *J. Chem. Soc.*, **1934**, 1797.

(4) A. W. Francis and A. J. Hill, *J. Am. Chem. Soc.*, **46**, 2498 (1924).

procedure as above that the nitrobiphenyl mixture consisted of 22% of the meta and of at least 19% of the para isomerides, the remaining 59% being made up of the ortho and para isomerides. The isomeric ratio from this competitive reaction agrees well with those obtained in the experiments with nitrobenzene as a sole component of the solvent. Taking the average of these figures and assuming the isomeric ratio to be $o:m:p = 58:23:19$, we estimate the relative reaction rate constants, which may be called reactivities, of the ortho, meta and para positions of the nitrobenzene nucleus to be 15, 5.8 and 9.8, respectively, relative to the rate constant at one position of the unsubstituted benzene nucleus taken as unity. Since the attack on the aromatic compound by the substituting entity, in whatever form it may react, is unimolecular with respect to the aromatic compound, the principle underlying our estimation is similar to that set forth by Ingold and Smith[5] and therefore it need not be described here. In the above calculation the ortho-para ratio was assumed to be 58:19 on the basis of the amount of the *p*-nitrobiphenyl isolated as a pure specimen, and the actual ortho-para ratio must therefore be smaller. In one experiment, *o*-nitrobiphenyl amounting to 21% of the total yield of nitrobiphenyls was isolated from the mixture of nitrobiphenyls from which para isomeride had already been separated. If this figure, in place of 58%, is taken to express the amount of the ortho isomeride actually formed, and consequently 56, in place of 19%, that of the para isomeride, the reactivities of the ortho and para positions will be altered to 5.4 and 30, respectively. The true reactivity lies, therefore, between 5.4 and 15 for the ortho, and between 9.8 and 30 for the para positions.

From the reaction of diazotized aniline in the presence of aqueous sodium hydroxide with nitrobenzene at 10° there was obtained a mixture of nitrobiphenyls in a yield of 15% based on the aniline used. The percentages of the isomerides formed were estimated to be 46 for the ortho, 18 for the meta and 36% for the para compounds, and the value for the para isomeride being based on the amount isolated as a pure specimen, the figures for the ortho and para nitrobiphenyls are subject to the same uncertainty as in the case described above.

When diazotized aniline was decomposed in a mixture of nitrobenzene and benzene (in a molar ratio of 1:30:60), biphenyl and a mixture of nitrobiphenyls were obtained in yields, based on the aniline used, of 5.2 and 12.7%, respectively. *m*-Nitrobiphenyl as titrated amounted to 20.3% of the total nitrobiphenyls and the para isomeride actually isolated to 34.4%. Assuming that the isomerides are formed in the ratio $o:m:p = 46:19:35$, the reactivities of the various positions of the nitrobenzene nucleus are estimated to be 6.6, 2.8 and 11 for the ortho, meta and para positions, respectively, relative to one position of the unsubstituted benzene nucleus.

Although ortho-para substitutions were observed in most cases of the formation of biaryls by the reaction under discussion, it was reported by some workers that the reaction between diazobenzene hydroxide and ethyl benzoate gave all of the possible isomeric ethyl biphenylcarboxylates,[6] and that in the decompositions of 4-nitrosoacetaminobiphenyl in both chloro- and bromo-benzene the substitution by the biphenyl group took place at the meta as well as at the ortho and para positions in the halogenobenzene nucleus.[7] The present results afford another example of the formation of the meta isomeride in a condiderable quantity along with the ortho and para isomerides. In view of these facts together with those that will be later reported from this laboratory it seems that the concomitant formation of all three isomerides in these reactions is the general rule. It is significant too that even the meta position of nitrobenzene was found to be more reactive than the benzene nucleus itself towards the substituting agent formed by the decomposition of nitrosoacetanilide and diazobenzene hydroxide.

## Experimental

**Materials.**—*n*-Nitrosoacetanilide was prepared according to O. Fischer[8] by introducing into a solution of acetanilide in glacial acetic acid nitrous fumes generated by reducing concentrated nitric acid with potato starch and by adding the dark green solution thus obtained to a large quantity of water. The precipitated nitrosoacetanilide was separated from the aqueous layer, washed several times with water and dried on a porous plate. Samples prepared in this way melted at 50-51° with decomposition. Benzene and nitrobenzene were purified by repeated fractionations through a Vigreux

(5) C. K. Ingold and M. S. Smith, *J. Chem. Soc.*, **1938**, 905.

(6) D. H. Hey, *J. Chem. Soc.*, **1934**, 1967.

(7) H. France, I. M. Heilbron and D. H. Hey *J. Chem. Soc.*, **1938**, 1364.

(8) O. Fischer, *Ber.*, **9**, 463 (1876).

column.

**Determination of *m*-Nitrobiphenyl.**—The amount of *m*-nitrobiphenyl in a mixture of isomeric nitrobiphenyls was determined by titration with a standard titanous chloride solution and subsequently with a bromide-bromate mixture. The procedure adopted was essentially that of Francis and Hill.[4] However, ethyl alcohol proposed by them as a solvent proved inadequate because it consumed bromine. After several experiments, it was found that glacial acetic acid which was refluxed with 2% of chromium trioxide and then twice distilled was a satisfactory solvent for the determination of nitrobiphenyls. The reduction was carried out in an atmosphere of carbon dioxide with boiling for fifteen minutes, and the bromination at 5 to 10°. Separate experiments with pure specimens of nitrobiphenyls showed that the number of bromine atoms taken up could be determined within the accuracy of 2%.

**Decomposition of Nitrosoacetanilide.**—When nitrosoacetanilide (0.5 g.) was dissolved in 27 cc. of nitrobenzene at 20°, decomposition took place evolving nitrogen without a perceptible rise in temperature. A set of twenty reaction mixtures similar to the above was prepared and allowed to react simultaneoulsly. After a few days, when the reaction had been completed, the reaction mixtures were combined and the nitrobenzene was removed under reduced pressure through a Vigreux column 30 cm. in length. The residue was subjected to further fractionation under reduced pressure, and 4.18 g. of a reddish-brown viscous oil passed over at 150 to 185° at 15 mm. of mercury. The oil deposited crystals of *p*-nitrobiphenyl, which on recrystallization from petroleum ether melted at 113° (yield 0.560 g.). The mother liquor of recrystallization and the remaining oil were combined and filtered through a column of activated alumina in order to remove a coloring matter. A further crop of *p*-nitrobiphenyl (47 mg.) was obtained by extracting the alumina with alcohol. The remainder was recovered as an oily mixture (3.05 g.), which was analyzed for the nitro group by titration with a standard titanous chloride and was found to contain only nitrobiphenyls. Subsequent titration with a bromide-bromate mixture showed that the mixture contained 28% of *m*-nitrobiphenyl.

In one experiment a fraction, which distilled after a substantial part of nitrobenzene had been removed, was reduced with tin and hydrochloric acid to remove nitrobenzene. Any traces of biphenyl which might have resulted from the dimerization of phenyl radicals were not detected.

The competitive reaction was accomplished by dissolving one gramme of nitrosoacetanilide in a mixture of 18.7 cc. of nitrobenzene and 32.7 cc. of benzene at 20°. Nine of such reaction mixtures were combined and worked up similarly to the experiment described above. That part of biphenyl which passed over with the vapor of nitrobenzene was recovered by converting the latter into aniline by treatment with tin and hydrochloric acid.

**Decomposition of Diazobenzene Hydroxide.**—To a stirred mixture of 430 cc. of nitrobenzene and diazotized aniline prepared from 13 g. of aniline, 33 cc. of concentrated hydrochloric acid (d. 1.17), 10.5 g. of sodium nitrite and 26 cc. of water there was added dropwise a solution of 14.4 g. of sodium hydroxide in 75 cc. of water in the course of thirty minutes, the temperature being kept at 8-10°, and stirring was continued for further five hours. The product separated from the aqueous layer was worked up as before. In the competitive reaction a mixture of 215 cc. of nitrobenzene and 373 cc. of benzene was used for the diazotized aniline from 6.5 g. of aniline. Products from two of such reactions were combined and worked up.

## Summary

The reactions of both *n*-nitrosoacetanilide and diazobenzene hydroxide with nitrobenzene give all three isomeric nitrobiphenyls, whereas the formation of only the ortho and para isomerides has been reported in the literature.[3] All the nuclear positions of nitrobenzene are more reactive than that of benzene towards the substituting phenyl radical.

The authors thank the Department of Education for a Grant-in-Aid for Scientific Research which has defrayed the expenses of this investigation.

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